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Denaturing RNA electrophoresis in urea polyacrylamide gels (Urea-PAGE) and semi-dry Northern Blot transfer

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1 *Works for me* dx.doi.org/10.17504/protocols.io.beemjbc6

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ABSTRACT

RNA is denatured in 8 M Urea and separated in a polyacrylamide gel electrophoresis (PAGE) based on its molecular weight. Using this method, fragments between 2 to 500 nucleotids can be separated. The migration of the RNA depends on the concentration of PAA: the higher the PAA percentage, the better the resolution of low molecular weight fragments and vice versa. We used 10 % PAA to separate fragments between 50 - 200 nucleotides in 10 ml mini-gels (10.5 x 10 cm) using the Hoefer SE260 Mighty Small system. Subsequently, RNA is transfered from the gel to a nylon membrane (Northern blotting) by using aTrans-Blot SD Semi-Dry Electrophoretic Transfer Cell (BioRad).

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KEYWORDS

RNA, Urea-PAGE, semi-dry transfer, Northern Blot

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GUIDELINES

- take care of safty precautions when working with PAA and ethidiumbromide
- wear gloves when handling RNA samples

MATERIALS TEXT

MATERIALS

ROTIPHORESE®Gel 40 (19:1) **Carl Roth Catalog #3030.1**

ROTIPHORESE®10x TBE-Puffer **Carl Roth Catalog #3061.1**

Ammonium persulfate (APS) **Sigma Aldrich Catalog #A3678-100G**

TEMED (NNN'N'-Tetramethylethylenediamine) **Sigma Aldrich Catalog #T9281-25ML**

Urea **Contributed by users**

2X RNA Loading Dye **Thermo Fisher Scientific Catalog #R0641**

3 MM Whatman Blotting paper **Carl Roth Catalog #0048.1**

Hybond N positively charged nylon membrane (Amersham) **Ge Healthcare Catalog #RPN303B**

Ethidium bromid stock solution 10 mg/ml **Thermo Fisher Scientific Catalog #15585011**

RiboRuler Low Range RNA Ladder **Fisher Scientific Catalog #SM1831**

For Urea PAGE

- 15 ml falcon tube
- serological pipettes
- plastic pasteur pipettes
- 1 x TBE buffer

10% APS:

- dissolve 1 g APS in 10 ml a. dest
- make 1 ml aliquots and store at -20 °C
- thawed samples are stored at 4°C and should be used within a few weeks

gel staining solution:

- 200 ml 0.5 x TBE, 20 µl of 10 mg/ml Ethidiumbromid (= 1 µg/ml final conc.)
- store at 4°C in the dark; staining solution could be reused several times

- Hoefer SE260 Mighty Small vertical electrophoresis system for 10 ml Mini-gel*s (or any other system* of comparable size)
- glassplates and notched alumina plates, 10 x 10.5 cm
- a pair of spacers 0.75 mm
- 10 well comb

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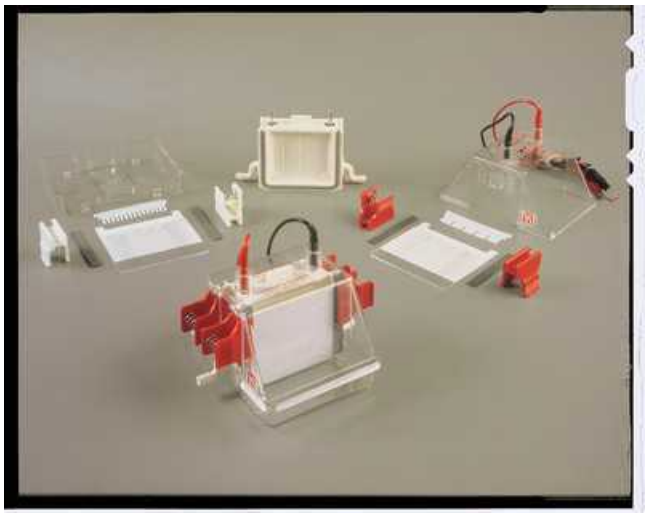

SE260 Mighty small vertical electrophoresis system

- SE245-gel-caster_description.pdf

For Northern Blotting

- Whatman paper
- Nylon membrane, positively charged
- clean plastic trays (e.g. square petridishes 120 x120 x 17 mm)
- serological pipette
- 0.5 x TBE buffer
- Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (BioRad)

SAFETY WARNINGS

always wear **vinyl** gloves while working with acrylamide!
Ethidiumbromid may cause mutations in the germ cells of humans! Wear protective gloves and clothes. The generation of waste should be avoided or minimized wherever possible. Empty containers or liners may retain some product residues. This material and its container must be disposed of in according to approved disposal technique.

preparation of Urea-PAA gel

1
- assemble the gel sandwich stack (see pdf in materials section); align plate bottoms with the spacers on a flat surface before sliding the sandwich in the casting clamp assembly and tithening the screws (finger tight to prevent breaking of the plates)
- place the sandwich into the caster, notched alumina plate facing to the back block; check that black silicone rubber gasket is clean
- two gels can be prepared in the caster; I suggest always to prepare two (in case that one sandwich is leaky); polymerized gel sandwiches can be stored for later use (see below)

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- prepare the gel according to the table below; 10% PAA is fine to separate fragments of 50 - 200 nt
- wear Vinyl gloves and goggles!

% PAA	4%	5%	6%	8%	10%	12%	15%	20%
40% AA/Bis (19:1)	1.0 ml	1.25 ml	1.5 ml	2.0 ml	**2.5 ml**	3.0 ml	3.74 ml	5.0 ml
Urea	5g							
10 x TBE	1 ml							
10% APS	80 µl							
TEMED	12 µl							
a. dest.	to 10 ml							

- weight 5 g urea and transfer to a 15 ml falcon tube
- add 2 ml 40% PAA and 1 ml 10 x TBE
- add a. dest. to a final volume of 10 ml
- heat at 37 °C with agitation until urea is completely solved (shake occasionaly by hand); this will take a while, you can use higher temperatures (e.g. 55 °C) to speed up the process
- let the solution cool down
- add 12 µl TEMED and mix by inverting the tube
- add 80 µl APS and mix by inverting the tube
- pour the gel quickly but carefully between the plates using a plastic pasteur pipette
- remove air bubbles by shortly lifting and pushing the caster to the table
- insert the comb immediately
- wait until gel gets solid (at least 1h)
- remove the gel from the caster and rinse the sandwich with destilled water to remove buffer and gel remnants
- thoroughly wash caster components in mild detergent and rinse with destilled water immediately after use
- proceed to step 2 or store the sandwich - wrapped in wet towel paper and foil - for later use at 4-8°C

run Urea-PAGE

2
- always wear gloves during all steps!
- thaw your total RNA samples and marker (RiboRuler Low Range RNA Ladder) on ice
- mix 5 µl total RNA (conc. 5 µg, dilute with RNase free water if needed) with 5 µl 2 x loading dye and incubate 5 min at 95°C (danatures RNA)
- marker: mix 3 µl LR-ladder with 2 µl RNase free water and 5 µl 2x loading dye and incubate at 70°C for 10 min
- snap cool on ice
- meanwhile assemble the electrophoresis system by inserting the upper buffer chamber in the lower buffer chamber; fix the sandwich to the upper chamber with the clamps, glass plate to the front
- optionally: put a well locating decal to the glass plate to mark the wells
- carefully remove the comb
- pour 1 x TBE buffer into the electrode chambers
- rinse the wells thoroughly with buffer by using a syringe (Hamilton), rinse again directly prior to sample application
- quickly spin down your samples in a microcentrifuge and carefully pipette samples into the wells
- close the safty lid and plug electrodes into a power supply
- run at constant current of 10 mA for 1/3 of the gel length, then increase to 20 mA for the rest of the run
- stop the run when the bromphenol blue dye (migrates at 12 nt in 10% Urea-PAGE) has just run out off the gel

visualization of RNA

3
-
-
- 3+6remove the lid and pour off the buffer
- remove the clamps and disassemble the gel sandwich: remove the spacers and carefully lift up the glass plate by using one of the spacers as a lever
- use the spacer to cut off the wells and the upper right corner of the gel (to mark the orientation of the gel)
- carefully transfer the gel to a clean tray and shortly rinse the gel in 0.5 x TBE
- incubate the gel 15 min in 25 ml 0.5 x TBE + 1 µg/ml Ethidiumbromid with gentle agitation in the dark (cover tray with aluminium foil)
- meanwhile wash the electrophoresis equipment in detergent, followed by dest water

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- collect the staining solution for re-usage and rinse the gel in 0.5 x TBE
- RNA is visualized in a transilluminator (Gel Doc Imaging System, BioRad), image area 13.4 x 10.0 cm, program nucleic acid- ethidiumbromide
- save a gel picture and put the gel back in 0.5 x TBE buffer, proceed to step 4

Northern Blot

4
- the electrophoretic transfer of the RNA to a nylon membrane is set up in a Trans-Blot SD Semi-Dry Electrophoretic Transfer cell (BioRad)
- cut a piece of nylon membrane (Hybond N+) and 8 pieces of 3MM Whatman paper in 1 x gel size, soak in 0.5 x TBE buffer
- humidify both electrode plates with 0.5 x TBE

Assembly of the blotting sandwich (bottom up):
- 4 layers of 3MM papers (1x gel size)
- nylon membrane (1x gel size)
- PAA-gel (remove bubbles with a serological pipette)
- 4 layers of 3MM papers (1x gel size)

- close the lid and plug electrodes into a power supply
- run at constant current of 2 mA/1 cm^2 gel for 45 min (= 180 mA for a 10.0 x 9.0 cm mini-gel)
- you can blot several sandwiches in one transfer
- after the transfer has finished remove the Whatmann paper and cut the membrane with a scissor along the edges of the gel to have exactly the same size as the gel (including cut off of upper right corner)
- optional: transfer the gel to 0.5 x TBE and check the transfer in the transilluminator - transfer was successful when you see fluorescence only in the uppermost part of the gel (high molecular weight fragments)
- transfer the membrane to sheet of towel paper; don't touch the membrane, use foreceps or grap it carefully at the edges
- put the membrane on the transilluminator table, RNA site down
- make a picture: approx. 0.1 s exposure to UV light (program nucleic acid - ethdiumbromide), image area same as for PAA-gel (10.0 x 13.4 cm); this is necessary for the accurate assignment of signals after hybridization with specific RNA probes!
- subsequently, cross-link the RNA to the membrane by exposure to UV-light for 120 sec (don't forget!)
- dry the membrane completely, mark the blot with a pencil and store it between the sheets of Whatman paper or proceed to hybridization with a specific probe

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