

Northern Blot (sRNA)

General Notes:

Practice RNase-Free technique:

- Use RNA-only pipet tips/tubes
- Use RNaseZap to clean counters and pipetmen
- Filter/sterilize solutions
- Use DEPC-H₂O or MQ-H₂O
- Keep RNA cold (4 °C or lower)

Needed Material:

- Mini-PROTEAN Tetra Cell
- Mini Trans-Blot Cell Assembly for Transfer
- Loading buffer (LBII (Ambion)- Glycerol, bromophenol blue, xylene cyanol, formamide)

- 10 X TBE (1 X TBE dilution):

- 108 g Tris Base
- 55 g Boric Acid
- 40 mL 0.5 M EDTA (disodium salt), pH 8.0
- In 960 mL of H₂O

- 20 X SSC (1 X and 6 X dilutions):

- 175 g NaCl
- 88 g sodium citrate
- 1 M HCl to adjust pH to 7.0
- Bring to 1 L with H₂O

- ULTRAhyb-Oligo BrightStar BioDetect Kit (Ambion)

Protocol (for detecting ~120 nt sRNA):

1. Prepare a 10% TBE-Urea gel:

To a beaker **add**:

- 3.3 mL 30% acrylamide (protogel)
- 1.0 mL 10 X TBE
- 2.136 mL water (MQ)
- 4.2 g urea

Cover with Parafilm and Incubate at 37°C or in hot bath (65°C) until urea dissolves. Then add

- 330 µL 1.6% APS (Needs to be less than 1 month old)
- 5 µL TEMED

Swirl to facilitate polymerization and then pour into gel casting frame.

Allow gel to **solidify** in a Mini-PROTEAN Tetra cell casting frame/stand for 45'. Gel can be stored overnight, wet, at 4 °C.

2. **Prepare RNA Samples:**

- a. **Prepare** RNA samples with equal volume of LBII, (try to load the same volume in each lane; adjust volume of RNA with DEPC-H₂O).
- b. **Heat** for 5' at 65 °C, **Pico-centrifuge**.
- c. Keep RNA cold (4 °C or lower).

3. **Pre-Run and Run Gel:**

- a. **Pre-Run gel** in 1 X TBE at 250 V for 20' in a Mini-PROTEAN Tetra cell tank.
- b. **Pipette** out gel debris from each lane before loading RNA samples.
- c. **Run gel** in 1 X TBE at 200 V for 45-60', (ensure dark blue loading dye runs off gel).

4. **Transfer Gel to Membrane:**

- a. **Equilibrate** sponges, filter paper and membrane in 1x TBE.
- b. **Prepare** gel sandwich (black, sponge, filter paper, 10% TBE-Urea gel, a 60 cm² membrane, filter paper, sponge, white).
- c. **Transfer** (black to black, clear to red) in Mini Trans-Blot Cell in 1 X TBE at 200 mA for 60'. Add a stir bar and ice block to cell and place on a magnetic stirrer.

5. **Wash Membrane and X Link:**

*Handle membrane **gently**, do not touch with fingers:*

- a. **Wash membrane** in 20 mL of 6 X SSC.
- b. **UV Cross link**.
- c. **Wash membrane** in 20 mL of 1 X SSC.

6. **Prehybridization and Hybridization:**

- a. **Pre-hybridize** (≥30') standard membrane size in 6 mL of ULTRAhyb-Oligo (Ambion) at 42 °C.
- b. Add ~600 ng of probe (5'-end labeled oligo) specific for RNA.
- c. Allow to **hybridize** overnight at 42 °C.

7. **Follow BrightStar BioDetect Kit to detect RNA the following day.**