Northern Blotting

Solutions

- 10X running buffer
  - 50 mM NaOAc
  - 0.2M MOPS pH 7.0
  - 10 mM EDTA
  - filter, store in dark at RT, pH to 7.0
- Formaldehyde gel
  - 0.9% agarose in 1X running buffer, and 1ml formaldehyde per 20 ml gel. (For 200 ml gel, dissolve 1.8 g agarose in 170 ml water, let cool in 65 deg C water bath, add 10 ml formaldehyde (37% stock), and 20 ml 10X MOPS running buffer).
- 1.5X loading buffer
  - 750 µl formamide
  - 75 µl formaldehyde (37%)
  - 150 µl 10X running buffer
  - 15 µl EtBr (5 mg/ml)
  - 10 µl ddH₂O
  - Best results if made fresh each time
- Loading Dye
  - Bromophenol blue in 35% glycerol and 1X running buffer
  - 20X SSC
    - 3M NaCl
    - 0.3M Na Citrate
    - pH 7.0

Procedure

1. Pour gel in hood.
2. Mix RNA (10µg) with 1.5X loading buffer (e.g. 10 µl RNA + 20µl LB)
3. Incubate 5 min at 65° C. Cool on ice.
4. Add 2 µl loading dye.
5. Pre-run gel at 5 V/cm for 5 minutes. Load samples and run. Mix buffers after 3 hours.
6. Wash gel 2X in water for 15 min, then 1X in 10X SSC for 15 minutes. Photograph gel under UV.
7. Cut nylon membrane (MSI 0.45 micron #N04HY00010) and several pieces of blotting (e.g. Schleicher and Schuell GB002) paper to the same size as the gel. Wet the nylon with dH₂O, then soak in 5x SSC.
8. Assemble sandwich
   1. Large sheet of plastic wrap
   2. Two pieces blot paper (precut to same size as gel) soaked in 20x SSC
   3. Gel (wells-side down)
   4. Presoaked nylon
   5. One piece blot paper soaked in 5x SSC
   6. 10-15 pieces of dry blot paper
   7. Wrap whole sandwich in the plastic wrap
   8. Place glass plate and weight on top and let transfer >3 hour
10. Can probe either with non-radioactive probe or with radioactive probe.