5/29/07
Original 8/30/89 from MSA-as in Gene Screen Manual

FORMALDEHYDE GEL ELECTROPHORESIS OF RNA & RNA Blot Hybridization

see Maniatis (1982), p.202 for reference and see Southern protocol in this manual

**Gel Preparation:** 3 g + 215 ml $H_2O$; microwave; stir

In fume hood: While stirring, add 25 ml 10 X MOPS (rt)
10 ml formaldehyde (freshly deionized with AG 501 X 8(D) resin)
Stir to mix well and pour into mold which has comb slightly raised fom normal position. Transfer to gel box before pulling out the comb.

Sample Preparation: Add appropriate amount (3 – 10 µg/lane) RNA to solution with final concentrations of 50% ultrapure formamide, 6% formaldehyde (deionized) 1 X MOPS. Heat 5 min at 65ºC. Add dye.

| | | | |
|---|---|---|---|
| 10 X MOPS Buffer: | 0.2 M MOPS | 41.8g free acid | )Recipe |
| For 1000 mL | 50 mM NaOAc | 4.1g | )from gene |
| | 10 mM EDTA | 20 ml of 0.5 M stock | )screen booklet |

-titrate to pH 7.0 with NaOH; store at 4ºC.

| | | | |
|---|---|---|---|
| 5 x Dye Solution | 25% glycerol | 10X = 50% | |
| | 0.2% bromphenol blue | 0.4% | |
| | 0.2% xylene cyanol | 0.4% | |

| | | | |
|---|---|---|---|
| 3 µl sample volume: | add in order shown | | |
| | 5.1 µl RNA | or | 8.1 µl RNA |
| | 3 µl 10 x MOPS | | 3 µl 10 X MOPS |
| | 12 µl formamide | | 12 µl formanide |
| | 3.9 µl formaldehyde | | 3.9 µl formaldehyde |
| | Heat 5', 65ºC | | Heat 5', 65ºC |
| | 6 µl 5 x Dye Solution | | 3 µl 10X dye |

(If your RNA is concentrated you can make a 20 µl sample by scaling down).

Run gel 4 – 5 hours at 100 V or ON at 40-50 V in 1 X MOPS

**Stain**

- Stain gel in 500 ml of $H_2O$ containing 50 µl of Ethidium bromide (10 mg/ml stock) 2 h, with gentle shaking
- Destain in $H_2O$ as necessary to take a picture (usually ON is convenient)
  NOTE: stained gels do not hybridize very well. Therefore, run duplicates, or do not stain.

**Blot**
Soak gel in 10 X SSC 2 x 15 min on reciprocal shaker
Blot overnight in 10X SSC; use 2 pieces of Whatman 3 MM paper on bottom of gel as wicks (plus 1-2 more pieces to size of gel) – see Southern procedure.
Wet gene screen or other membrane in $H_2O$ and then soak in 10 x SSC for 5'. It is important to remove gel gap from the membrane.

**Irradiation**
The filter is marked with a felt pen and washed vigorously in 6 X SSC. Place the filter (still wet) on a glass plate and cover it with seran wrap.
Irradiate the filter with 1.6 kJ/m² (1200 µW/cm²) (with a relatively new trans-illuminator at 260 nm this is approximately 5 minute exposure at a distance of 15 cm., but check date of most recent calibration on the illuminator).
Irradiate through Saran wrap – not through glass.
Rinse the filter in distilled water 2 -3 times.

Hybridization and wash procedure from Church & Gilbert (PNAS **81**; 1991-1995, (1984))

**Hybridization**

Hybridization buffer: 7% SDS
0.5 M $NaPO_4$, pH 7.2
1% Bovine Serum Albumin (Sigma, fraction V)
Note: 1 M $NaPO_4$, pH 7.2 is made with 134 grams of $Na_2HPO_4$ (7 $H_2O$) plus 4 ml of 85% $H_3PO_4$ per liter. This is stock solution for hybridization and washing solutions.
Note: This recipe is what I use, but it does not give IM NaP (KH 4/17/91).

Rehybridize the filter for 10 minutes or longer in approximately 0.1 ml hybridization buffer/cm² at 65ºC.
Note: I prehybridize and hybridize at 68º (KH 4/17/91)
Remove buffer and add .05 ml/cm² of fresh buffer with probe. Remember to denature probe!!
Hybridize 12 – 16 hours at 65ºC.
Wash blot – 6 x/15'/65ºC/shaking/100-200 ml of 1% SDS in 40 mM $NaPO_4$, pH 7.2.
Can wash several blots together without cross contamination. (After hybridization, rinse each filter briefly in a fresh aliquot of 200 ml wash solution (rt); then pool filters in 200 ml for 6 x washes as above).

See southern protocol for exposure.