Northern Blotting

Solutions

RNase-Free water

Prepare DEPC 0.1%(v/v) in Milli-Q water. Mix well, autoclave.

10X MOPS/EDTA

0.5M MOPS pH 7.0
0.01M EDTA pH 7.5 – 8.0

For 500mlL: 53.325g MOPS
10mL 0.5M EDTA pH 8.0
Bring up to 500mL with Milli-Q
pH to 7.0 with NaOH
Filter through 0.2µM filter
Store wrapped in aluminum foil

Formaldehyde/Formamide

89µL 37% formaldehyde
250µL deionized formamide

Buffer A

294µL 10X MOPS/EDTA
106µL RNase-free water

Gel Loading Buffer

322µL Buffer A
5mg xylene cyanol FF
5mg bromocresol green
400mg sucrose

Mix well, add:
178µL 37% formaldehyde
500µL formamide

Sample Preparation

1. Speed vac (on "medium" drying rate) 10µg total RNA, or 1-2µg poly A RNA. The lids of the tubes should be open, and their mouths covered with perforated parafilm.
2. Add 5µL Buffer A and resuspend RNA.
3. Add 12µL formaldehyde/formamide solution and mix.
4. Incubate samples at 68°C for 10 minutes and place on ice.
5. Add 4µL gel loading buffer and mix.

Gel Preparation and Electrophoresis

1. Wash tray, comb and electrophoresis apparatus with 10% SDS and rinse thoroughly with Milli-Q. Alternatively, soak them in RNase free water overnight, or *at least* while performing the subsequent steps.
2. Prepare a 1% agarose gel in 1X MOPS/EDTA buffer and cool to 60°C.
3. In a fume hood add 9mL 37% formaldehyde to 41 ml of the cooled agarose. Mix and cast gel. Allow gel to polymerize for at least 45 minutes.
4. Pre-run gel at 40 volts for 45 minutes in 1X MOPS/EDTA buffer.
5. Load samples and run gel at 40 volts for small gels (80 volts for large gels).
6. Allow gel to run until bromophenol blue dye reaches ~ 1cm from the bottom.

Transfer

1. After electrophoresis, wash gel in 20X SSC for 20 minutes.
2. Pre-wet membrane in Milli Q water or in 2X SSC.
3. Transfer gel using Vacugene apparatus. The order of components, starting from the bottom, is: screen, membrane, mask, gel. The membrane should be ~ 1 cm larger than the opening of the mask all around. Fill with 20X SSC (10X SSC works equally well) until gel is covered. Transfer at 70—80 cm/H_20 pressure for 2 hours.
4. After transfer, rinse membrane in 2X SSC and crosslink ("Auto Cross Link" function of the Stratalinker).

Prehybridization

Pre-hyb solution (final concentrations):	Volume of stock solutions required:
55% formamide	5.5 ml formamide
5X SSC	2.5 ml 20X SSC
1x Denhardts	200 µl 50X Denhardts
50mM phosphate buffer, pH 7.0	2.5mL 0.2M phosphate buffer, pH7.0
250µg/mL ssDNA (fish sperm DNA)	250 µl 10 mg/ml ss DNA

-Alternatively, 500µL 50X Denhardts and 1.5mL 0.2M phosphate buffer, pH 7.0 (other components the same) will work equally well.
-For 100mL 0.2M phosphate buffer (pH 7.0), mix 57.7mL 1M Na_2HPO_4 and 42.3mL 1M NaH_2PO_4

-Incubate the membrane in the pre-hyb solution for *at least* 4 hours at 42°C (overnight, if preferred).

Hybridization

Hyb solution (final concentrations):	Volume of stock solutions required:
55% formamide	3.3mL formamide
5X SSC	1.5mL 20X SSC
1x Denhardts	120µL 50X Denhardts
20mM phosphate buffer, pH 7.0	600µL 0.2 M phosphate buffer, ph 7.0
10% dextran sulphate	1.2mL 50% dextran sulphate
250µg/mL ssDNA (salmon sperm DNA)	150µL 10mg/mL ssDNA

-For labeling and counting of the probe, see protocol for Southern blotting.
-Prepare hyb mixture without the probe. Place the membrane in the container; add the hyb solution, and finally, the probe. Incubate overnight at 42°C.

Washing

Two alternatives methods (work equally well):

Method 1
-Remove membrane from hyb solution and rinse 3x at room temperature with 2X SSC+0.1% SDS solution.
-Wash the membrane 2x 30 minutes, in 2X SSC+0.1% SDS, at 42°C while shaking.

Method 2
-Remove membrane from hyb solution and rinse 3x at room temperature with 2X SSC+0.1% SDS solution.
-Wash the membrane 4x 5 minutes in 2X SSC+0.1% SDS at room temperature while shaking.
-Wash the membrane 2x 15 minutes in 0.1X SSC+0.1% SDS at 50°C while shaking.
-Regardless of the method used, check counts with Geiger in between the longer washes, especially if the probe is not particularly "hot". If the membrane is still "hot" overall, continue washing. Otherwise, stop and expose the membrane to a phosphorimager screen for at least 4 hours.

Stripping

-Place membrane in 0.1X SSC+0.1% SDS, 2x 30 minutes, at 85°C with vigorous shaking.