

## Northern blotting protocol

(submitted by Ross Alexander, Beggs' Lab)

### Original reference for basis of northern blotting procedures:

Alwine JC, Kemp DJ, Stark GR (1977). "Method for detection of specific RNAs in agarose gels by transfer to diazobenzyloxymethyl-paper and hybridization with DNA probes". Proc. Natl. Acad. Sci. U.S.A. 74 (12): 5350-4. Procedures and buffer composition taken from Ausubel *et al.*, (1997). "Current protocols in molecular biology". Greene Publishing Associates, New York and Wiley-Interscience, New York and Chicester.

### Solutions

Buffer	Components	
10X TBE	0.89M Tris base, 0.89M boric acid, 20 mM EDTA	
10X BPTE	100 mM PIPES, 200mM Bis-Tris, 10mM EDTA pH 8.0	DEPC treated or filtered
20X SSC	3M NaCl, 0.3M sodium citrate	pH adjusted to 7.0 with 1 M HCl
20X SSPE	3.6M NaCl, 0.2M NaH <sub>2</sub> PO <sub>4</sub> , 20mM EDTA	pH adjusted to 7.4 with NaOH
SES1	0.5M sodium phosphate pH 7.2, 7% (w/v) SDS, 1mM EDTA	Store at 30°C
Formamide loading dye	96% (v/v) formamide, 20 mM EDTA ph 8.0, some BPB and XCB	
Glyoxal reaction mixture	6 ml DMSO, 2 ml deionised glyoxal, 1.2 ml of 10X BPTE buffer, 0.6 ml 80% glycerol, 20 µl of 10 mg/ml ethidium bromide,.	stored in small aliquots at -70°C

### Denaturing PAGE of RNA (For RNA smaller than 1.2kb).

Denaturing polyacrylamide-urea gels (0.2 – 1.0 mm thick) prepared using sequagel 6 or 8 (National Diagnostics) for 6% or 8% (19:1) gels respectively.

- 20ml monomer solution
- 100ml casting solution
- 0.8% APS (10%) per 100ml

Polymerised gels were pre- run in a vertical tank (Cambridge Electrophoresis, Model No. EV200) containing 1X TBE for 30 min to 1h at 25 W with a metal plate to distribute heat evenly

RNA samples were mixed with an equal volume of 2X formamide loading dye and heated for 2 min at 95°C.

Samples snap chilled in ice water and collected at bottom of tube by centrifugation. (Optional)

Samples loaded into rinsed wells and gel run for required length of time (45 – 120 min).

## **Electrophoresis of glyoxylated RNA through agarose gels (RNA larger than 1.2 kb)**

Five µg (upper limit) of total RNA in 2 µl mixed with 10 µl of glyoxal reaction mixture and heated for 1h at 55°C.

Samples snap chilled in ice water and collected at bottom of tube by centrifugation.

Samples loaded on a horizontal 1.2% agarose gel.

Run in 1X BPTE overnight at 55 V in an EMBL H135 gel tank until bromophenol blue nears the bottom of the gel.

Load non RNA containing lanes with standard DNA loading dye containing BPB and XCB as a reference for migration.

## **Transfer of RNA to membrane**

### **Method 1**     *After polyacrylamide gel electrophoresis*

(All sponge pads pre-soaked in 0.5X TBE, 3MM paper and Hybond -N, wet with 0.5X TBE prior to use)

Peel gel of onto Whatman 3MM paper.

Place this onto very wet (TBE) sponge pad.

The pre-wetted Hybond-N membrane then placed directly on top of the gel. Roll test tube gently over surface to remove any air bubbles.

Place whatman 3MM on top of this.

To complete sandwich another sponge pad placed on top of this.

The frame was closed, placed in tank and RNA transfer performed in 0.5X TBE in water-cooled Bio-RAAd Trans-Blot cell (also used for protein transfer) at 60V for 1h.

Blotted RNA then crosslinked to membrane in a UV Stratalinker 1800 (stratagene) using the 'autocrosslink' function.

X2 RNA side up, X1 reverse side.

**Method 2** *After electrophoresis of glyoxylated RNAs through agarose gels*

Gel washed in deionised water and incubated in 75 mM NaOH for 20 min.

Gel then incubated in Tris/ NaCl (0.5M Tris-HCL pH 7.0, 1.5 M NaCl) for 15 min.

Followed by two washes in 6X SSC.

Gel stacked on top of Whatman 3MM paper on a bridge suspended over a container with 6X SSC with filter paper hanging into the solution.

Hybond-N<sup>+</sup> membrane soaked in 6X SSC placed on top of gel (taking care not to leave any bubbles).

Three layers of soaked 3MM filter paper followed by three layers of dry 3MM were then placed on top.

Stacks of hand towels and a weight placed on top, thus ensuring constant pressure.

Parafilm used around the gel to ensure the towels only soaked up liquid passing through the gel and membrane.

Transfer carried out overnight.

Blotted RNA then crosslinked to membrane in UV Stratalinker as above.

**End-labelling of oligonucleotides**

Oligonucleotides labelled in a 10 µl reaction mixture by T4 polynucleotide kinase (PNK).

Oligonucleotide	10pmol/µl	1 µl	
10X PNK buffer		1µl	
T4 PNK	10U/µl	0.5µl	37°C for 45 min.
[γ- <sup>32</sup> P] ATP	3000 Ci/mmol	2µl	
Distilled H <sub>2</sub> O		5.5µl	

End-labelled oligonucleotides were then purified through a 2µ filter, using SES1 buffer to elute.

## **Hybridisation**

Crosslinked membrane pre-hybridised in 50 ml SES1 at 37°C for 30 – 45 min.

Pre-hybe solution decanted off and purified end-labelled oligos in 50 ml SES1 buffer added.

Incubate at 37°C (or probe optimal temperature) overnight in a shaking oven or Rotating Hybe oven.

Decant probe and wash 2X for 15 min in 6X SSPE buffer at 37°C.

Wash 2X for 30 min in 6X SSPE (pre-warmed to 42°C).

Wrap blot in cling film and develop at -70°C.

## **Stripping blots**

To strip, a boiling solution of 0.1 % SDS was poured onto the membrane and incubated on a platform shaker for 10 min.

Repeat 3 times or until no counts detected.

Then reprobe as above.