High-resolution Northern blot preparation using the glyoxal/dimethyl sulphoxide method of RNA denaturation

Amersham Biosciences supplies a range of matched systems and reagents for high-sensitivity and convenient blotting experiments. The Rediprime® II DNA Labelling System combined with Redivue® [α-^{32}P] dCTP-labelled nucleotides and the autoradiography products provides an efficient and convenient method for the high-sensitivity detection of probes for Northern blots. To ensure consistency of results, an equally efficient and convenient method of blot production should be used. The method described here utilizes glyoxal and dimethyl sulphoxide (DMSO) as denaturants and offers a safer and more convenient alternative to other commonly used methods.

The use of glyoxal/DMSO denaturation eliminates the need for fume hoods. No wash treatments are required prior to blotting, and the use of glyoxylated RNA results in narrower bands following electrophoresis.

Successful Northern analysis depends on the quality of the reagents used as well as having undegraded RNA samples. To avoid contamination with RNases, use sterile disposable plastics wherever possible. Glassware may be decontaminated by baking at 180 °C overnight or incubating in 0.2% (v/v) diethylpyrocarbonate (DEPC) followed by autoclaving or baking. Some plastics are also compatible with DEPC treatment.

Products used

HE 99X Max Submarine Electrophoresis Unit	80-6061-57
EPS 301 Power Supply	18-1130-01
VacuGene™ XL Vacuum Blotting Unit	80-1266-24
VacuGene XL Vacuum Blotting Pump	
115 V	80-1265-14
220 V	80-1265-15
Hybond™-N+ nylon transfer membrane	RPN2020B
Rediprime II DNA Labelling System	RPN1633
Redivue [α-^{32}P] dCTP	AA0005
Hyperfilm™ MP	RPN6H

Solution tables

10× Nucleic Acid Loading Dye Mix

(0.4% bromophenol blue, 0.4% xylene cyanol, 25% w/v Ficoll™ 400, 10 ml)

Bromophenol blue	0.4%	40.0 mg
Xylene cyanol	0.4%	40.0 mg
Ficoll 400	25% (w/v)	2.5 g

Add approximately 8 ml of distilled water; mix to dissolve and make up to a final volume of 10 ml. Store at room temperature for up to 3 mo.

10× MOPS Buffer

(0.20 M MOPS, 80 mM NaAc, 10.0 mM EDTA, pH 7.0, 1 litre)

MOPS [3-(N-morpholino)-propanesulphonic acid, FW 209.3]	0.20 M	41.2 g
NaAc·3H$_2$O (FW 136.1)	80 mM	10.9 g
Na$_2$EDTA·2H$_2$O (FW 372.2)	10 mM	3.7 g

Add approximately 800 ml of nuclease-free water; mix to dissolve. Adjust to pH 7 with sodium hydroxide and make up to a final volume of 1 000 ml. Filter-sterilize and store at room temperature, protected from light.

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Protocol

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Reagent preparation

Glyoxal oxidizes very rapidly to produce glyoxylic acid which causes RNA fragmentation. On receipt, deionize the solution to a neutral pH using a mixed bed ion exchange resin. Aliquots can then be stored at -20 ºC for up to 6 mo and thawed as required. Once thawed, aliquots of glyoxal should be used only once and the unused solution discarded.

Prepare a 10× stock of MOPS buffer. Filter-sterilize the solution and store at room temperature, protected from light. Properly stored, the solution is stable for up to 3 mo. Do not use the solution if it appears yellow in colour, however.

Agarose gels should be prepared to the required volume and concentration in 1× MOPS buffer.

Dissolve the agarose in nuclease-free water before adding 10× MOPS stock. Do not add ethidium bromide to glyoxal gels, because the dye will react with glyoxal, changing its spectral properties.

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Denaturation of RNA

Prepare the deproteinized RNA samples using the following table:

	Volume (µl)	Final concentration
RNA	×*	
10× MOPS buffer	1.5	0.5 ×
Deionized glyoxal	5	1 M
DMSO	15	50%
Nuclease-free water	5.5 – ×	
TOTAL	27	

*Values determined by the volume of RNA solution.

Incubate the samples at 50 ºC for 60 min. Following incubation, add 3 µl of 10× nucleic acid loading buffer. Mix and load onto the agarose gel.

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Electrophoresis

Electrophoresis is carried out using the HE 99X Max Submarine Electrophoresis Unit in 1× MOPS buffer at 3–4 V/cm gel. The low voltage is important to prevent the formation of a pH gradient.

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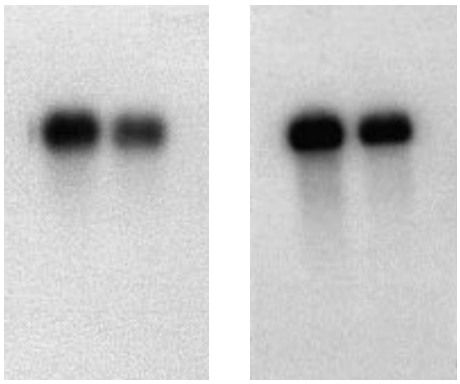
Transfer

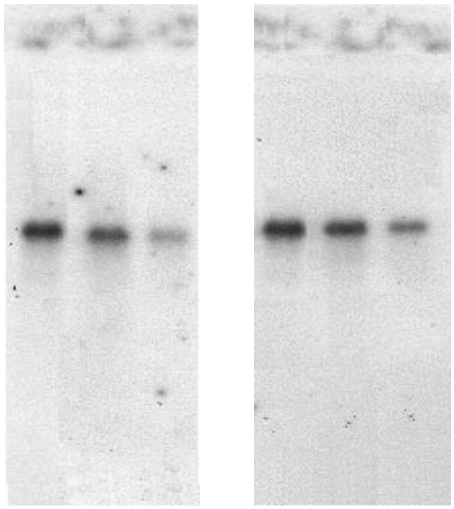
Transfer RNA to the membrane, using either a standard capillary blotting protocol or the VacuGene XL Vacuum Blotting System. If vacuum blotting, an alkali hydrolysis step is not required prior to transfer, and no pre-treatment is required with either procedure to remove glyoxal from the gel.

Results



Formaldehyde RNA treatment | Glyoxal RNA treatment

Fig 1 Comparison of the formaldehyde and glyoxal RNA denaturing systems with Hybond-N+ nylon membrane. Human skeletal muscle mRNA, 1 µg and 0.5 µg loadings on Hybond-N+ membrane. Human GAPDH probe labelled with Redivue [α-^{32}P] dCTP using Rediprime II DNA Labelling System. Probe used at 2×10^6 cpm/ml of aqueous phosphate plus casein hybridization buffer.



Formaldehyde RNA treatment | Glyoxal RNA treatment

Hybridization was carried out at 65 ºC for 16 h. Hyperfilm MP was exposed for 16 h with intensifying screens at -70 °C.

Fig 2 Comparison of the formaldehyde and glyoxal RNA denaturing systems with Hybond-N+ nylon membrane. Human liver mRNA, 4 µg, 2 µg, and 1 µg loadings on Hybond-N+ membrane. Human p53 probe labelled with Redivue [α-^{32}P] dCTP using Rediprime II DNA Labelling System. Probe used at 2×10^6 cpm/ml of aqueous phosphate plus casein hybridization buffer. Hybridization was carried out overnight at 65 °C. Hyperfilm MP was exposed overnight with intensifying screens at -70 °C.

Conclusions

The preparation of high-quality Northern blots is the key to successful hybridization experiments. Glyoxal/DMSO denaturation is a quick and convenient protocol which is adaptable to both capillary and vacuum blotting methods. When coupled to the Rediprime II DNA Labelling System and Redivue nucleotides, the added convenience and improved safety features make it the method of choice for high-resolution Northern blots.

References

Farrell, R. E., in *RNA Methodologies, A Laboratory Guide for Isolation and Characterisation*, chapter 8, Academic Press, Inc., 1993.

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