Volume 17 Number 22 1989 Nucleic Acids Research

Immediate visualization of blotted RNA in Northern analysis

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Submitted September 18, 1989

Modern protocols for Northern analysis discourage staining of agarose gels with ethidium bromide (EtBr) after electrophoresis, citing an earlier report (1) which suggested a major loss in hybridization signal when blotting EtBr-stained RNA. We arrived at a different conclusion using our protocol. **Fig. a** shows a photograph of a gel after electrophoresis of EtBr-stained RNA (lanes 1-7) and unstained RNA (lanes 9-15). Apart from providing information on the quality of RNA size-separation, on RNA integrity, on the amount of RNA loaded, and on the position of the 28S and 18S bands, staining of RNA revealed a major advantage when the same gel was vacuum-blotted onto a nylon membrane. During UV-fixation the blotted RNA, even in the 0.3 µg lane, emitted a strong fluorescence signal which could easily be photographed (**Fig. b**). Thus the quality and quantity of RNA transfer onto the support material can for the first time be assessed immediately after blotting. When the blot was hybridized with a ^{32}P labeled γ-actin probe using a standard formamide protocol, the hybridization signals (**Fig. c**) of the EtBr-stained samples were slightly weaker (11 %-18 %; representative of 9 experiments). In our view this moderate decrease in signal is clearly outweighed by the critical information available when using stained RNA.

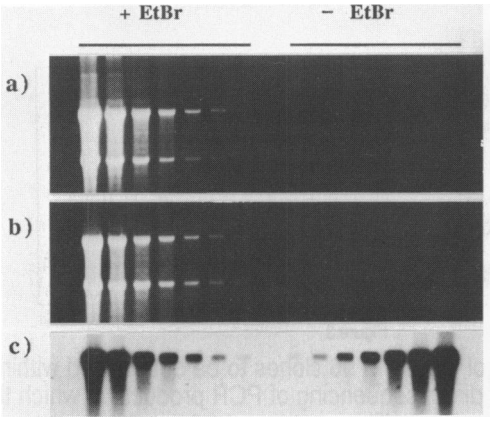

Figure: Titrated amounts (20 µg - 0.3 µg) of EtBr-stained (lanes 1-7) and unstained (lanes 9-15) RNA were electrophoresed on a 1.2 % agarose gel. **a)** Photograph of the gel after electrophoresis, **b)** photograph of the blot after transfer, during UV-fixation, **c)** autoradiography after hybridization with a ^{32}P labeled γ-actin probe.

METHODS: Titrated amounts of total RNA (20 µg-0.3 µg) from the human histiocytic line U-937 were vacuum-dried, resuspended in 50 µl of sample buffer (1X MOPS buffer, 6.54 % formaldehyde, 50 % formamide), then 10 µl of loading buffer (1 mM EDTA pH 8.0, 0.25 % bromophenol blue, 0.25 % xylene cyanol, 50 % glycerol) were added. To some samples EtBr was added (2 µl of 0.5 mg/ml stock). The samples were heated to 55 °C for 15 min. (heating with EtBr present substantially increases the fluorescence signal without adverse effects on the hybridization signal), briefly quenched on ice and electrophoresed on a 1.2 % agarose gel containing 1.1 % formaldehyde for 3 1/2 hours at 70 V with buffer recirculation (1X MOPS = 0.02 M 3-morpholino-propanesulfonic acid, 0.005 M sodium acetate, 0.001 M EDTA, pH 5.5 -7.0). The gel was vacuum-blotted (VacuGene, Pharmacia LKB, Bromma, Sweden) at -60 cm H_2O over 4 hrs. onto a nylon-membrane (Zetabind, AMF Cuno, Meriden, CT) using 20X SSC for transfer. For UV-fixation (4 min.) and photography a transilluminator (TM-36, UVP Inc., San Gabriel, CA) was used. Autoradiographies were eveluated by densitometry.

REFERENCE: 1. Thomas P. S. (1983) In Wu, R., Grossman, L., and Moldave, K., (eds.), Methods in Enzymology, Vol. 100, Academic Press, N.Y., pp. 255-266.

