Northern hybridization: rapid and simple electrophoretic conditions

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Received January 27, 1993; Revised and Accepted April 22, 1993

Current methods for Northern blot analysis to determine mRNA abundance in tissues are tedious and utilize toxic chemicals. The requirement for chemicals of high purity, free of ribonucleases, and the lability of RNA, in comparison to DNA, are additional factors preventing wider use of the technique. For Northern blot analysis, RNA is generally denatured with glyoxal (1) or formaldehyde (2) prior to electrophoresis in agarose gels. The gels require extensive washing for ethidium bromide or acridine orange staining to localize major RNA species or fragments used as size markers. These manipulations are time-consuming and contain several steps where ribonucleases can be introduced, which can result in failure of the experiment. In addition, these techniques do not lend themselves to easy visualization of the degree of RNA migration during electrophoresis or allow the experimenter to determine the efficiency of transfer of the RNA following blotting. This report outlines a rapid and simple method which requires just a 5 minute denaturation of RNA samples in loading buffer and allows the experimenter to monitor the integrity and migration of major RNA species and size markers during electrophoresis (Figure 1A). In addition, the stained RNA is easily detected following blotting onto a nylon or nitrocellulose membrane, thus allowing the experimenter to determine the efficiency of transfer of the RNA from the gel (Figure 1B). The technique is summarized in the following three easy steps.

(a) Rapid denaturation of RNA. In a 1.5 ml sterile Eppendorf tube 10 µl of the RNA (1 to 10 µg) dissolved in sterile H2O is mixed with 2 µl of sterile 6 x loading buffer [6 x loading buffer = 0.25% (w/v) bromophenol blue, 0.25% (w/v) xylene cyanol, 30% (w/v) glycerol, 1.2% SDS, 60 mM sodium phosphate (pH 6.8)]. The mixture is incubated at 75°C for 5 min followed by immediate loading of the sample onto a submarine gel. When

Figure 1. Analysis of RNA migration with and without glyoxal denaturation and hybridization following transfer to a nylon filter. Between 1 and 3 µg of different total RNA preparations from Trypanosoma brucei brucei, ILTat 1.1 (4), were heat denatured and electrophoresed on a 1.4% agarose gel as described (A1, lanes 1 to 4). The gel was visualized on a UV-transilluminator following electrophoresis for 1 hour (A1) and 3 hours (A2). Lane M contains 1 µg of RNA low molecular weight size markers (BRL), 1.77, 1.53, 1.28, 0.78, 0.53, 0.40, 0.28 and 0.16 kb. (B1) 1 µg of RNA low molecular weight size markers (BRL) (lane M) and 5 µg of total RNA from Trypanosoma congolense, clone IL 3000 (5) (lane 1), were resolved on a 1.4% agarose gel and visualized by UV light. RNAs were electroblotted from the gel to a Nytran membrane (Schleicher and Schuell) in 25 mM sodium phosphate buffer, pH 6.8, as recommended by the manufacturer. The filter was then placed on a UV-transilluminator and photographed to determine the efficiency of transfer of the RNA (B2). BPB indicates the position of the bromophenol blue dye which fluoresces strongly on the filter. (C) shows differences in the mobility of 5 µg of total T. b. brucei RNA (lanes 1 and 2) and 1 µg of RNA high molecular weight size markers (BRL), 9.5, 7.5, 4.4, 2.4, 1.4 and 0.24 kb (lanes 3 and 4), following denaturation by heating to 75°C alone (lanes 1 and 3) or by glyoxal treatment (lanes 2 and 4). (D) demonstrates the integrity of the RNA and hybridization efficiency following transfer to a nylon membrane. Poly(A)+ enriched RNA (1 µg) from actively-dividing long slender (lane 1), non-dividing stumpy bloodstream forms (lane 2) and in vitro-derived procyclic insect forms (lane 3) of T. b. brucei ILTat 1.1 were electrophoresed as described, transferred to a Nytran membrane and fixed to the membrane by UV light using a Stratalinker (Stratagene, USA). The filter was hybridized with a β-tubulin gene probe, labelled with α-32P-dCTP using a Prime-It kit (Stratagene, USA), followed by washing and autoradiography.

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analyzing many samples, the denatured RNA can be placed on ice before loading on a gel.

(b) Preparation of the gel. The agarose is melted by boiling in 10 mM sodium phosphate buffer, pH 6.8, containing 1 μl of 10 mg/ml ethidium bromide per 100 ml of buffer, then cooled to 60°C and poured.

(c) Conditions of electrophoresis. The gel is electrophoresed at 3 to 7 V/cm in 10 mM sodium phosphate buffer, pH 6.8, containing 1 μl of 10 mg/ml ethidium bromide per 100 ml of buffer. Because the buffering capacity of the electrophoretic buffer is relatively weak due to its low ionic strength, constant recirculation of the buffer is maintained to prevent the formation of an undesirable pH gradient which can lead to degradation of the RNA during electrophoresis. The electrophoresis can be interrupted at any time and the migrating RNA in the gel visualized with medium-wave UV light to verify the migration and integrity of the RNA (Figure 1A). Following electrophoresis, the RNA can be directly transferred to a nylon membrane (Nytran, Schleicher and Schuell or Hybond N+, Amersham) using standard techniques for blotting or electro-transfer (2) and fixed to the filter by a short UV light exposure or NaOH treatment (as recommended by the suppliers). The filter-bound RNA is easily visualized by placing the filter on a UV-transilluminator (Figure 1B) and the position of co-migrating size markers can be marked with a non-water-soluble ink pen. Using this method, the migration of RNA molecules during electrophoresis is inversely related to the log10 of the size, although there is a relative change in the RNA mobility in comparison to glyoxal treated RNA (Figure 1C). Staining of the RNA by ethidium bromide does not interfere with its hybridization to radiolabelled probes or cause high background signals on the filter (Figure 1D). The hybridization signal is comparable to that using glyoxal (Figure 2A and 2B) and can be improved by washing the gel for 10 min in 7% (v/v) formaldehyde following electrophoresis (Figure 2C). The filters can also be rehybridized several times, following removal of a probe, without loss of signal (data not shown).

The combination of this gel-electrophoresis technique with the single-step RNA purification method of Chomczynski and Sacchi (3), together with UV cross-linking for Nytran or NaOH treatment for Hybond N+, should make Northern blot analysis a faster and more convenient technique in the future.

REFERENCES


This is ILRAD publication number 1065.