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A simple procedure for gel electrophoresis and Northern blotting of RNA

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Electrophoresis through agarose is frequently employed during the characterisation of RNA, particularly in its analysis through subsequent Northern blotting. The electrophoretic behaviour of RNA is, however, influenced by secondary structure. Therefore, in order to force co-migration of identical RNA species, electrophoresis is routinely performed under denaturing conditions. A number of different denaturants are employed for this purpose, most commonly formaldehyde (1–3), but also, to a lesser extent, glyoxal/dimethyl sulfoxide (4–6) and, most infrequently, mercuric hydroxide (7). There are, however, many disadvantages associated with the use of each of these denaturating agents (8). As volatile liquids exhibiting varying degrees of toxicity, all impose containment and handling restraints on the operator, e.g., the use of fume hoods. This is particularly the case with mercuric hydroxide, which, although the most efficient of these denaturants, is the most unpopular due to its extreme toxicity. Other problems greatly inconvenience the operator. Thus, for instance, the liver carcinogen formaldehyde is readily oxidised and great care must be taken in its storage. Furthermore, its complete removal from the gel matrix employed, a prerequisite for subsequent Northern blotting, requires extensive destaining in DEPC-treated water to get rid of formaldehyde. A similar difficulty also hinders the use of glyoxal/dimethyl sulfoxide, although the major drawback to the use of these particular denaturating agents is that the required running buffer has an extremely low buffering capacity, necessitating buffer changes or the use of a buffer recirculation system.

As an alternative to the above, we report here the use of guanidine thiocyanate (Fig. 1). This compound represents a far less toxic denaturating agent, dispensation of which, being a non-volatile solid, does not require the use of fume hoods. Using a freshly prepared 1 M stock solution, it may be conveniently added to molten agarose immediately prior to casting of the gel. At a final concentration of 20 mM, its presence is sufficient to maintain electrophoresed RNA in a denatured form whilst protecting it from the action of RNase. Furthermore, electrophoresis may be undertaken in standard TBE buffer, and, unlike other denaturants, the RNA may be stained with ethidium bromide *in situ* without recourse to further pre-treatment of the gel. This allows gel electrophoresis to be interrupted at any time and the migrating RNA visualised under UV light to verify the integrity of the sample and ascertain the distance migrated.

Thereafter, in contrast to formaldehyde, there is no necessity to remove the guanidine thiocyanate prior to Northern blotting. Thus, RNA can be transferred directly to the nylon membrane being employed with no further pre-treatment. The presence of ethidium bromide ensures that the filter bound RNA can be easily visualised by placing the filter on a UV-transilluminator, allowing the position of co-migrating size standards to be marked, for reference purposes, with a permanent ink pen.

We have successfully used guanidine thiocyanate in the analysis of RNA from a number of different *Clostridia* species, including *C.butyricum*, *C.acetobutylicum* and *C.tetanomorphum*. Its use as the denaturating agent provides considerable advantages over other commonly employed chemicals, not least of which are improvements in operation times, safety aspects and the facility to directly stain samples with ethidium bromide.

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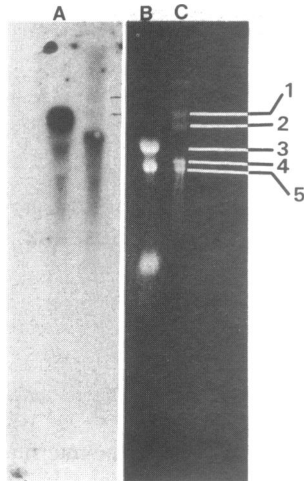

Figure 1. Characterisation of *Clostridium botulinum* NCTC 2916 RNA. The right-hand panel represents an ethidium bromide stained, 1.2% (w/v) agarose gel containing 20 mM guanidine thiocyanate on which has been electrophoresed 3 μg of *C.botulinum* RNA (lane B) and 1 μg of RNA markers (lane C), of size: 1, 7.4 kb; 2, 5.3 kb; 3, 2.8 kb; 4, 1.9 kb; 5, 1.6 kb (Boehringer Mannheim). The RNA of lanes B and C were subsequently transferred to a nylon membrane and hybridised with a ^{32}P-labelled, *botA*-specific DNA fragment. The left-hand panel is the resultant autoradiograph, where lane A corresponds to lane B. Gels were prepared by adding 0.5 ml of a freshly prepared 1 M solution of guanidine thiocyanate and 2 μl ethidium bromide (10 mg/ml) to 100 ml of molten (60°C) agarose immediately prior to pouring into a casting tray pre-soaked (2 h) in 1% (w/v) SDS. Electrophoresis was at 8 V/cm in TBE buffer (90 mM Tris–HCl, pH 8.0, 90 mM sodium borate, 2 mM EDTA) containing ethidium bromide. Following electrophoresis, the gel was soaked in 0.05 M NaOH for 30 min, and the RNA transferred to a nylon membrane (Hybond N$^+$, Amersham) according to the manufacturer's instructions. Blots were pre-hybridised [in 10 ml of 0.2 M NaH_2PO_4, 0.3 M Na_2HPO_4, 0.5 M EDTA and 7% (w/v) SDS] for 30 min before the addition of a ^{32}P-labelled 0.8 kb fragment specifying an internal portion of the *botA* gene (9). Label was incorporated using a Megaprime kit (Amersham International) using the manufacturer's protocol. Hybridisation was for 4 h at 50–60°C, and excess probe removed by washing [three times in 2× SSC, 0.1% (w/v) SDS at 50–60°C]. Blots were briefly air dried and autoradiographed at –70°C against Kodak film. RNA was isolated from late exponential phase cells (from a 10 ml of culture) suspended in 500 μl AE buffer (50 mM sodium acetate pH 5.3, 10 mM EDTA Na_2). Cells were lysed by adding 50 μl of 10% (w/v) SDS and, following a brief vortex, an equal volume of AE-equilibrated phenol and then incubating at 65°C for 5 min. After rapid chilling (2 min in dry ice/ethanol), the mixture was re-extracted with an equal volume of phenol:chloroform (1:1), subjected to centrifugation (10 000 *g* for 5 min at 4°C), and the RNA in the aqueous phase precipitated at –70°C by the addition of 0.1 vol 3 M sodium acetate (pH 5.3) and 2.5 vol ethanol. Subsequently pelleted RNA (10 000 *g* for 10 min at 4°C) was washed with 80% (v/v) ethanol, resuspended in 50 μl RNase-free DNase I buffer (100 mM sodium acetate, 5 mM $MgSO_4$, pH 5.0) and incubated with 20 U DNase I (Boehringer Mannheim Ltd) at 37°C for 1 h. Thereafter, the RNA was re-extracted twice with phenol:chloroform (1:1), and precipitated as before and re-suspended in 50 μl RNase-free distilled water. Yields equated to ~1 μg/μl. For electrophoresis, RNA was denatured by mixing a 10 μl sample aliquot with 10 μl formaldehyde and 5 μl formamide, heating at 90°C for 5 min, and then adding 3 μl of 10× loading dye (50% glycerol, 1 mM EDTA, pH 8.0, 0.4% bromophenol blue, 0.4% xylene cyanol).