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Non-radioactive detection of m_3G capped RNAs by immunoblotting

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Small nuclear RNAs (snRNAs) are present in all eukaryotic cells and with the exception of U6 snRNA contain a 5′ trimethylguanosine cap structure. We describe here the use of a monoclonal antibody to detect 2,2,7 trimethylguanosine capped RNAs in Northern blots of total RNA preparations. This procedure does not require ^{32}P-labelled RNA (1) or ^{35}S-labelled antibody (2) for immunoprecipitations and has the additional advantage of employing non-radioactive detection.

Nuclear extracts were prepared as described (3). Total RNA from nuclear extract was prepared by digestion with 100 μg/ml proteinase K in 100 mM Tris–HCl pH 7.5, 150 mM NaCl, 1% SDS, 12.5 mM EDTA, followed by phenol extraction and ethanol precipitation. Total RNA from mammalian cells was prepared using NP40 lysis as described (4).

RNA samples were fractionated on 10% 19:1 acrylamide gels containing 8 M urea. After staining with ethidium bromide the gel was soaked in TBE and electroblotted onto Genescreen (DuPont) membranes using TBE as transfer buffer. The RNAs were fixed on the membrane by UV crosslinking (5).

The filters were blocked for 2 hrs at room temperature in 10% dry milk in PBST (PBS [0.14 M NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.0] containing 0.05% Tween 20). After blocking, the 2,2,7 trimethylguanosine antibody (100 μg/ml) was added to a final concentration of 5 μg/ml. The antibody against 2,2,7 trimethylguanosine (6) is available from Oncogene Science. After incubation for 2 hrs at room temperature the filters were washed four times in PBST for 10 minutes each and secondary peroxidase-conjugated anti-mouse antibody (Jackson, Immuno Research) (dilution 1:10000) was added in PBST, 10% dry milk. After 1/2 hour the filters were washed six times in PBST for a total time of 1/2 hour. Finally, the filters were overlaid with chemiluminescence detection solution (Amersham). The signal was usually visible after less than 10 minutes of exposure on Hyperfilm (Amersham).

This method allows the rapid and non-radioactive detection of RNAs that contain 2,2,7 trimethlyguanosine caps. As can be seen in Figure 1, no cross-reactivity with mRNAs, which contain m_7G caps, or with rRNAs in total RNA preparations is observed. We use this method routinely to check the integrity and relative concentration of snRNAs present in nuclear extracts from different tissues and cell lines. This detection format should also be useful to determine whether novel RNAs contain 2,2,7 trimethlyguanosine caps.

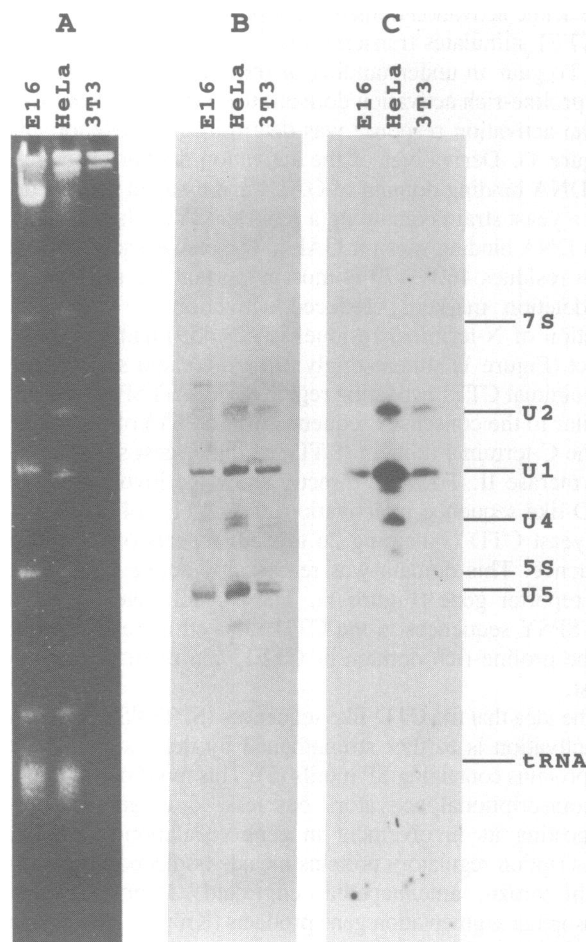

Figure. Detection of m_3G capped RNAs by immunoblotting. The identity of the RNAs is indicated on the right side. U2 snRNA from brain differs in sequence and modifications from other U2 snRNAs [7] which accounts for the lower mobility. **A.** 5 μg of total RNA from embryo rat brains (embryonic day 16, (E16)), nuclear extract from HeLa cells (HeLa NE) and nuclear extract from mouse 3T3 cells (3T3 NE) were fractionated on a 10% acrylamide/8 M urea gel and stained with ethidium bromide. **B.** The gel was electroblotted and probed with an anti-2,2,7 trimethylguanosine antibody. The exposure time was 10 minutes. **C.** A parallel blot was probed with ^{32}P-end labelled oligomers complementary to U1, U2 and U4 snRNA. The hybridization was carried out at 40°C in 500 mM sodium phosphate buffer, pH 7.0 and 5% SDS. The filter was washed twice for 15′ in 2× SSC/0.5% SDS and exposed overnight. The hybridization pattern confirms the identity of the RNAs in (B).

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