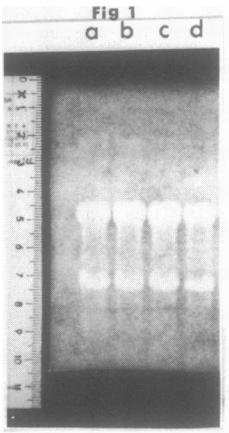
Volume 16 Number 5 1988 Nucleic Acids Research

A rapid and accurate method for quantitating total RNA transferred during Northern blot analysis

Nathalie Denis, Daniel Corcos, Jacques Kruh and Alain Kitzis

Institut de Pathologie Moléculaire Unité Associée CNRS, Faculté de Médecine, Chochin-Port-Royal, 75014 Paris, France
Submitted December 3, 1987

The accuracy of the quantitation of a given species of messenger RNA is dependent on the equality of the amounts of total RNA in the various samples transferred on the filter after Northern blotting. For this purpose a labelled probe for a messenger which is assumed to be present at the same level in all the samples is often used. However, one can never be sure that the level of this ubiquitous messenger will never vary depending on the cell type.
One the other hand, ribosomal RNA is a better reflect of total RNA, since it represents more than 80% of it . Hybridization with labelled RNA probes can then be used but it is usually performed only after having probed for the messenger of interest.
In this work , we show that, after staining of RNAs on agarose gel with ethidium bromide and transfering on nylon filter, the transferred ribosomalRNA can be visualized by submitting the filter to the U.V light . A photograph of the filter allows the determination of their relative amounts in the different studied samples (Fig 1).We have controlled that this method interferes neither with RNAs transfer, as it was previously reported with nitrocellulose filters (1) , nor with RNA hybridization with a labelled probe (Fig 2). It also allows an accurate determination of the size of the studied mRNAs. This method is not only much faster but also more accurate than when labelled RNA probes are used.



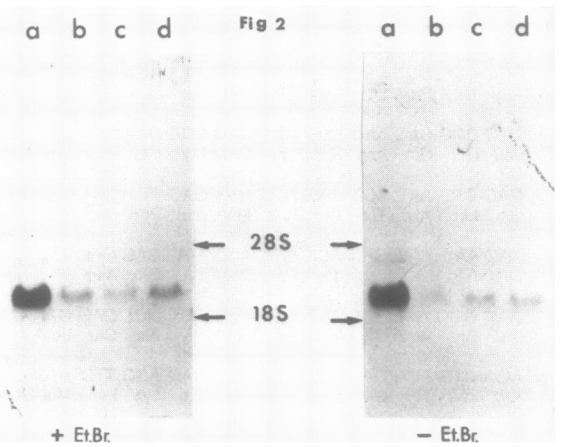


FIGURE 1
Northern blot analysis of c-myc mRNA in L6 cells under different conditions of growth and differentiation .RNAs were isolated from proliferative L6 cells (a) and from L6 cells cutured in 2% FCS for 12 h (b) , 24 h(c), or 48 h(d) . One of the two gels was ethidium bromide stained and both gels were treated for nylon filter transfer. The filter corresponding to the ethidium bromide stained gel was photographed under U. V light (Fig 1) and both filters were hybridized with a c-myc exon 3 probe washed, and exposed for the same time (Fig 2).

REFERENCES

1. Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982) Molecular cloning (Cold Spring Harbor Laboratory).