A novel, sensitive fluorometric staining technique for the detection of DNA in RNA preparations

Raman Mocharla, Hanna Mocharla and M.E.Hodes

Department of Medical Genetics, School of Medicine, Indiana University, Indianapolis, IN 46223, USA
Submitted November 13, 1987

RNA samples must be checked for DNA contamination before hybridization to an appropriate probe. This in turn requires a sensitive method for detection of DNA in the presence of RNA. Here we present a simple method based on the specificity of a fluorochrome, Hoechst 33258-DNA interactions (1), for direct visualization of nanogram amounts of DNA in the presence of large quantities of RNA in agarose gels. DNA from normal human blood cells and RNA from human and dog tissues were isolated as described (2,3). DNA and RNA samples were analyzed in both DNA slab gels (0.7% agarose) and RNA gels containing urea or formaldehyde (4,5). DNA slab gels were stained with H33258 (1 $\mu\text{g}/\text{ml}$ in 0.1 M potassium phosphate buffer, pH 7.2 containing 3 M NaCl). To visualize RNA, without further treatment these agarose gels were counterstained in ethidium bromide (EB; 1 $\mu\text{g}/\text{ml}$ in distilled water). Acid-urea or formaldehyde gels were washed in distilled water for 1 and 4-6 hr respectively before they were stained with H33258 and counterstained with EB. Gels stained with H33258 and EB were photographed in UV light with a Polaroid MP-4 system on 107C film, (f4.5; 1/15-1 sec exposure) through a blue (Kodak CG40B) or orange (Kodak 149-5589) filter, respectively (Fig. 1). The method detected as little as 0.003 μg of human DNA in agarose gels. The dye stained selectively small amounts of DNA even in the presence of large quantities of RNA (DNA:RNA=1:800; DNA: yeast tRNA = 1:3333). Overall, the procedure is sensitive, relatively simple, reproducible, highly selective for DNA and applicable not only to DNA gels but also to RNA gels. The differential staining technique allows analysis of electrophoretically fractionated DNA and RNA species on the same gel.

Fig. 1. Demonstration of selective staining of DNA by H33258. DNA and RNA samples were fractionated in 0.7% agarose gels, stained with H33258(A) and EB(B). Lanes: 1, 0.05 μg DNA + 5 μg lung RNA; 2, 5 μg human lung RNA; 3, 0.05 μg human genomic DNA

REFERENCES

1. Cesarone, C.F., et al. (1979) Anal. Biochem. 100:188-197.
2. Madisen, L., et al. (1987) Am. J. Human Genet. 27:279-290.
3. Chirgwin, J.M., et al. (1979) Biochemistry 18:5294-5299.
4. Davis et al. (1986) Basic Methods in Molecular Biology, Elsevier Sci. Publ. Co., New York, N.Y.
5. Woo, S.L.C. et al. (1975) J. Biol. Chem. 250:7027-7039.

ACKNOWLEDGMENTS:

This investigation was supported by grants from the S.R. Noble Foundation, Inc. to R.M. and the National Dental Research Institute (5T 32DE 07043).