Guillemette, J.G. & Lewis, P.N. Detection of subnanogram quantities of DNA and RNA on native and denaturing polyacrylamide and agarose gels by silver staining. Electrophoresis 4, 9...

ARTICLE *in* ELECTROPHORESIS · FEBRUARY 1983

Impact Factor: 3.03 · DOI: 10.1002/elps.1150040112

2 AUTHORS:

Joseph Guy Guillemette
University of Waterloo
87 PUBLICATIONS **1,975** CITATIONS
SEE PROFILE

Peter N. Lewis
University of Toronto
53 PUBLICATIONS **2,202** CITATIONS
SEE PROFILE

Short communication

Joseph G. Guillemette and
Peter N. Lewis

Department of Biochemistry,
University of Toronto

Detection of subnanogram quantities of DNA and RNA on native and denaturing polyacrylamide and agarose gels by silver staining

A silver staining method is described for detecting subnanogram amounts of DNA and RNA on polyacrylamide and agarose gels. The key step for increased sensitivity and linearity is an initial soaking of the gel in a 0.1 % solution of cetyltrimethylammonium bromide (CTAB). Agarose-containing gels can be silver stained after treatment with formaldehyde. The methods described are comparatively rapid, extremely sensitive (≅ 150 pg of DNA) and inexpensive (0.4 g of AgNO per 25 ml of slab volume).

The use of silver staining to detect small amounts of nucleic acids on polyacrylamide gels has recently been reported [1–3]. The methods used in these papers are not applicable to agarose-containing gels and, in two cases [1, 2], give staining intensities which vary with the size of the nucleic acid fragment for the same quantitiy of nucleotides. We report here modifications to protein silver staining methods [4, 5] which increase the sensitivity and linearity of stained nucleic acid bands and are also applicable to agarose-containing gels.

All electrophoretic separations were made on 19.5 × 16 cm, 0.8 mm slab gels in BRL vertical electrophoresis cells, type V 16. Chemicals used were of reagent grade or better. Nondenaturing gels contained 5 % acrylamide with 10 mM Tris-cacodylate-EDTA, pH 8 [6]. Samples were made to 20 % glycerol and 0.01 % Bromophenol Blue. Electrophoresis was for 1.5 h at 200 V with the above buffer. Denaturing gels contained a composite of 2.5 % acrylamide (acryl:bis ratio of 20:1) and 0.5 % agarose with 2.2 M formaldehyde, 20 mM phosphate, pH 7.5 [7]. Samples were made to 50 % formamide, 2.2 M formaldehyde and 20 mM phosphate, pH 7.5, and were heated to 60 °C for 5 min prior to loading. Electrophoresis was carried out for 2 h at 200 V with the above buffer but without the formamide. HAE III restriction enzyme and pBR322 DNA [8] were purchased from Boehringer-Mannheim. *E. coli* total RNA was obtained from Miles. Total RNA was isolated from mouse brain by standard methods [9]. Chicken polynucleosomes were prepared according to the method of Olins *et al.* [10] and analyzed by two-dimensional electrophoresis [11].

Silver staining was accomplished as follows: (A) Polyacrylamide gels: After electrophoresis a gel was transferred directly to a square plastic freezer container (21 cm) with a 1-cm hole in the bottom which facilitated rapid and gentle solution changes. The hole was plugged with a rubber stopper during staining. Considerable care was taken to avoid touching the gels with fingers or gloves during the transfer from the glass plate to the plastic container or during the various washes. The gel was sequentially soaked with gentle agitation for 1) 30 min in 0.1 % CTAB, 2) 30 min in distilled water, 3) 15 min in 0.3 % ammonia, 4) 15 min in an ammoniacal silver solution, 5) rinsed rapidly (< 15 s) with distilled water and finally 6) developed with 2 % sodium carbonate containing 0.02 % formaldehyde. All solution volumes were 250 ml. The ammoniacal silver was prepared just before use by mixing 2 ml of 20 % silver nitrate solution, 1 ml of 1 N NaOH and 1 ml of 30 % ammonia and 246 ml of distilled water. Development usually took 15 min and was arrested by pouring off the developer and adding 250 ml of 0.25 % nitric acid. After 15 min the stained gel was washed with distilled water and stored in a plastic freezer bag (Dow). (B) Agarose and composite polyacrylamide-agarose gels: Agarose and composite gels containing formaldehyde were soaked after electrophoresis with gentle agitation three times in 0.1 % CTAB for 20 min each and three times in distilled water for 10 min each. The repetitive washes are necessary to remove the formaldehyde which would otherwise cause premature reduction of the silver. The washed gel was soaked for 15 min in 0.2 % silver nitrate without ammonia, rinsed rapidly (< 15 s) with distilled water and developed as above with the same developer. Agarose and composite gels which did not contain formaldehyde were soaked in 0.1 % CTAB for 30 min, washed in distilled water for 30 min, soaked in 4 % formalin for 30 min, washed with distilled water three times for 10 min each and finally stained with silver and developed as above. (C) Proteins and nucleic acids on the same gel: 15 % Tris-sodium dodecyl sulfate (SDS)-glycine polyacrylamide slab gels (0.8 mm) were soaked after running for 30 min in 50 % methanol, 10 % acetic acid, 30 min in distilled water, 30 min in 0.03 % ammonia, 15 min in ammoniacal silver, washed 10 min with distilled water and then developed as above.

(A) Polyacrylamide gels: Fixation of nucleic acid in polyacrylamide gels with a cationic detergent such as CTAB prior to silver staining improves the sensitivity and linearity considerably as is shown in Fig. 1. Densitometer scans of selected tracks with and without CTAB for the gels in Fig. 1 are shown in Fig. 2. These results demonstrate that CTAB increases the sensitivity by a factor of three and markedly improves the linarity of staining. We estimate that bands of double stranded DNA containing as little as 150 pg of nucleotides are readily detectable. It is likely that the CTAB improves the sensitivity and linearity by causing aggregation of the DNA [12] in the gel matrix and by masking the effects of variations in base composition by coating the DNA with the hydrophobic cetyl group. Allen [13] has noted that an

Correspondence: Dr. P. N. Lewis, Medical Sciences Bldg., University of Toronto, Toronto, Ontario M5S 1A8, Canada

Abbreviation: CTAB: Cetyltrimethylammonium bromide

0173-0835/83/0102-0092$02.50/0

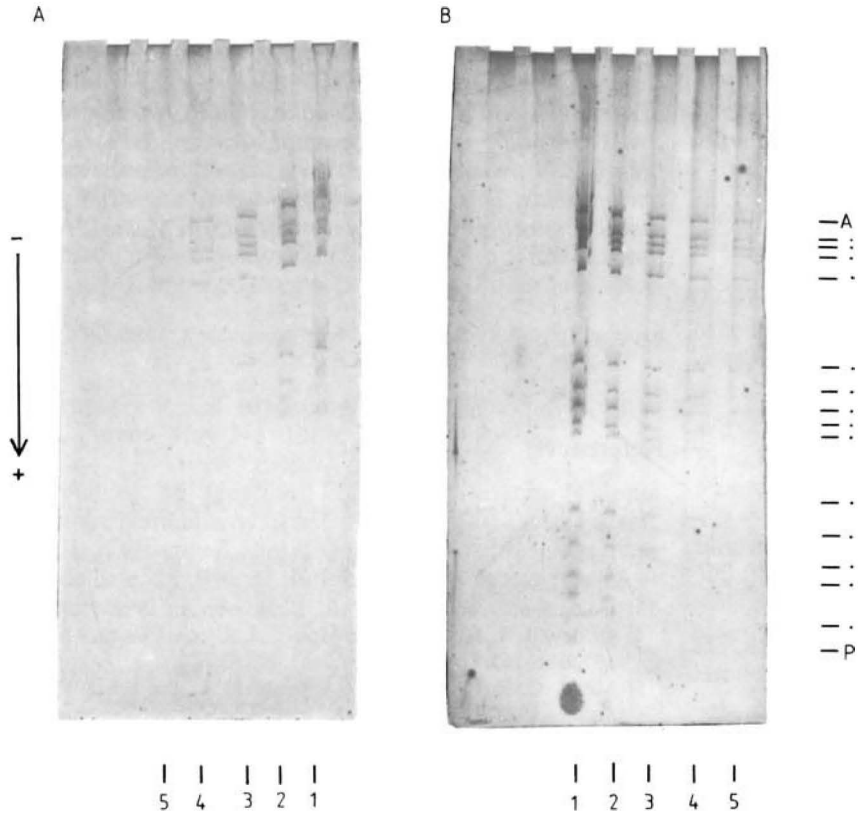

Figure 1. Silver staining of HAE III – pBR322 fragments with and without CTAB. Tracks 1–5 were loaded with 20, 10, 6, 3, and 1 ng respectively of a HAE III digest of pBR322; (A) without CTAB soak and (B) with soaking in CTAB. The 5 % polyacrylamide gel contained 10 mM tris-cacodylate 0.7 mM EDTA, pH 8. Fragment sizes visible on the gel are 587, 540, 458, 434, 267, 213, 192, 184, 124/123, 104, 89, 64, 57 and 51 base pairs (8) indicated as A through P respectively.

increase in the sodium to ammonium ratio tends to increase silver deposition and consequently decreases the specificity of the silver staining of proteins. The sodium to ammonium ion ratio in our procedure is roughly double the 0.034 value used in his investigation. Perhaps this increase might explain the improved linearity of staining for various DNA fragments.

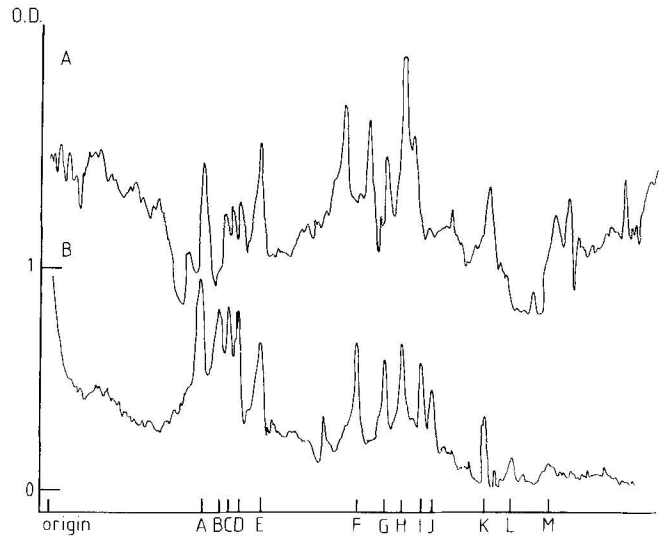

Figure 2. Evidence for the linearity of silver staining with decreasing DNA fragment size. Track 2 in both control (A) and CTAB (B) soaked gels shown in Fig. 1 was scanned with a densitometer. The recorder gain for the control gel was increased by a factor of three relative to the CTAB gel so as to obtain approximately equal peak intensities. The identity of the various bands are indicated A through M and have the sizes given in the legend to Fig. 1. Band K contains a doublet of 123/124 base pairs with 570 pg of nucleic acid.

(B) Agarose containing gels: We, as well as others [2], have found that silver staining protocols applicable to polyacrylamide gels give considerable surface staining and high backgrounds when agarose is present in the gel or when they are part of a composite gel. This effect can be greatly reduced by treatment of the gel with an aliphatic aldehyde prior to silver staining. For composite agarose gels we have found that a variation of the method of Oakley *et al.* [4] works well when formaldehyde is used in place of glutaraldehyde and is further improved with the CTAB step. This is shown in Fig. 3 for total RNA from *E. coli* and mouse brain run on a denaturing composite polyacrylamide-agarose gel. The sensitivity with CTAB is comparable to that found for the DNA samples used in Fig. 1. When non-denaturing gels are used, the agarose-containing gel is soaked in formaldehyde prior to silver staining. In this way contaminating amino groups in the agarose, which presumably cause the high background, are modified to prevent silver binding. We note that treatment of the formaldehyde-soaked gel with ammonium hydroxide solutions restores the high background. This is consistent with the formation of a Schiff base between amino groups in the agarose and the formaldehyde.

(C) Proteins and nucleic acids on the same gel: One of the requirements mentioned above for successful staining of nucleic acid is the rapid rinsing of the silver-soaked gel just before development. Prolonged (> 2 min) washing of the silver-soaked gel with distilled water removes much of the bound silver and subsequent development of these washed gels results in a negative image for nucleic acids. The same is not true for proteins and consequently it is possible to distinguish proteins from nucleic acids on the same gel. Protein bands give a positive brown image while nucleic acids produce a band which is markedly lighter than the general

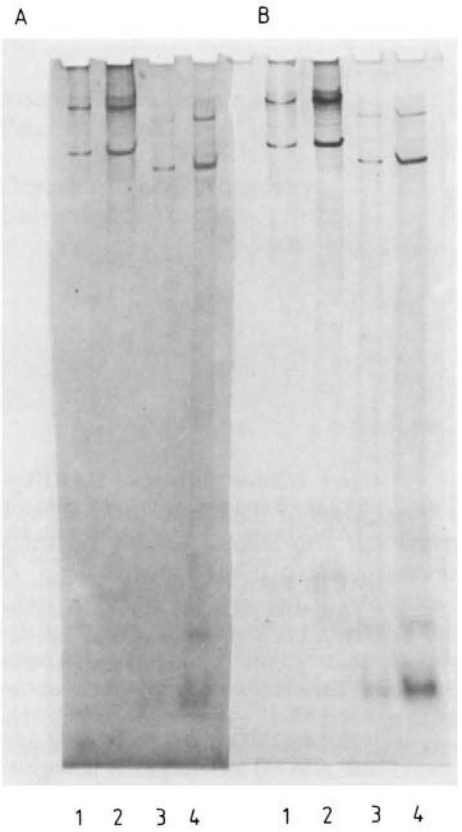

Figure 3. Silver staining of RNA samples run on composite polyacrylamide-agarose gels with and without CTAB. Both gels contain 2.2 M formaldehyde. Samples loaded in both (A) – without CTAB and (B) – with CTAB were: 1) and 2) 100 and 500 ng of total mouse brain RNA, respectively, and 3) and 4) 100 and 500 ng of total *E. coli* RNA, respectively.

background. This was tested using chicken polynucleosomes run in two dimensions (result not shown). A post silver wash of 10 min in distilled water was found to be optimal for the 0.8 mm slabs used here. This procedure allows visualization of both protein and nucleic acids on the same gel and should be useful, for example, in the analysis of nucleosomes and other nucleoprotein complexes.

We are grateful to Dr. G. Poirier at Sherbrooke University for communicating to us that the Oakley et al. [4] silver staining procedure for proteins reduces the background on agarose gels and to Drs. T. Boulikas and R. Hancock at the Swiss Institute for Experimental Cancer Research for preprints of their papers on silver staining of proteins and nucleic acids. This work was supported by the Medical Research Council of Canada and the Ontario Mental Health Foundation.

Received July 30, 1982; in revised form November 8, 1982

References

[1] Somerville, L. L. and Wang, K., *Biochem. Biophys. Res. Commun.* 1981, *102*, 53–58.
[2] Boulikas, T. and Hancock, R., *J. Biochem. Biophys. Meth.* 1981, *5*, 219–228.
[3] Goldmann, D. and Merril, C. R., *Electrophoresis* 1982, *3*, 24–26.
[4] Oakley, B. R., Kirsch, D. and Morris, N. R., *Anal. Biochem.* 1980, *105*, 361–363.
[5] Merril, C. R., Goldman, D., Sedman, S. A. and Ebert, M. H., *Science* 1981, *211*, 1437–1438.
[6] Maniatis, T., Jeffrey, A. and van de Sande, H., *Biochemistry* 1975, *14*, 3787–3793.
[7] Peacock, A. and Dingman, C., *Biochemistry* 1968, *7*, 668–674.
[8] Sutcliffe, J. G., *Nucleic Acids Res.* 1978, *5*, 2721–2728.
[9] Chirgwin, J., Przybyla, A., MacDonald, R. and Rutter, W., *Biochemistry* 1979, *18*, 5294–5299.
[10] Olins, A., Carlson, R., Wright, E. and Olins, D. E., *Nucleic Acids Res.* 1976, *3*, 3271–3291.
[11] Lewis, P. N., *Can. J. Biochem.* 1977, *55*, 736–746.
[12] Bellamy, A. R. and Ralph, R. K., *Methods in Enzymol.* 1967, *12B*, 156–160.
[13] Allen, R. C., *Electrophoresis* 1980, *1*, 32–37.