Method to Reduce the Quantity of Ethidium Bromide Required to Stain DNA in Agarose Gels

BioTechniques 23:780-782 (November 1997)

Ethidium bromide-agarose gel electrophoresis is an extensively used procedure for the analysis of DNA; it has been reported to be sensitive to as little as 50 ng of DNA (7). The standard methods for staining gels involve adding 0.5 µg/mL ethidium bromide to both the agarose gel and the running buffer or soaking the gel in a solution of ethidium bromide after electrophoresis has been performed (6). Intercalation of the ethidium bromide molecule between DNA bases causes fluorescence under ultraviolet (UV) light (6). Although fluorescence is much greater for the DNA-ethidium bromide complex, unbound ethidium bromide is responsible for the background fluorescence sometimes seen on agarose gels (6). Ethidium bromide is a suspected carcinogen and a known mutagen (1,4). Time-consuming deactivation procedures are therefore necessary before disposal of this substance (2,3,5).

We describe a simple method that reduces the quantity of ethidium bromide necessary to stain DNA in agarose gels. This modified procedure consists of adding 0.25 µg/mL ethidium bromide to the agarose gel only and then performing electrophoresis as usual.

To demonstrate the sensitivity of this modified staining procedure, a series of bacteriophage λ DNA (Sigma Chemical, Poole, England, UK) dilutions (16–500 ng) were prepared in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA and mixed with an appropriate volume of 6× loading buffer (4% sucrose, 0.25% bromophenol blue). DNA samples were then electrophoresed on submarine 1% agarose gels in TBE running buffer (90 mM Tris-HCl, 90 mM boric acid, 1 mM EDTA, pH 8.0) at 70 V for 2 h. Gels were photographed on a 302-nm UV light transilluminator after staining with either the standard method or our modified procedure. Figure 1 shows the typical data obtained with each protocol. The results when 0.5 µg/mL ethidium bromide was added to both the gel and the running buffer are shown in Panel A. The results obtained from adding 0.25 µg/mL ethidium bromide to the gel only are shown in Panel B. To quantitatively compare the sensitivity of each method, gel photographs from two separate experiments were analyzed with the Phoretix 1-D gel analysis software package (Phoretix International, Newcastle-Upon-Tyne, England, UK). Figure 2 shows the relative fluorescence intensity of each amount of DNA on gels stained with the two protocols. Values are expressed as a percentage of that obtained for the 500-ng DNA band on each gel. With both methods, DNA could be detected at levels as low as 16 ng, although the background fluorescence was slightly greater on gels stained with the standard protocol (Figure 1A). The apparent small difference in relative intensity obtained with the two procedures (Figure 2) was not statistically significant. We have routinely used the modified procedure described here to stain DNA fragments over a size range of 170 bp–50 kb and have found its sensitivity to be comparable to, or slightly better than, the normal procedure over the whole size range. Lower concentrations of ethidium bromide (0.1 µg/mL) were tested but found to be less effective at staining DNA fragments smaller than 2 kb (not shown). This is presumably because there was insufficient ethidium bromide present to fully saturate the available sites in the DNA.

Our data show that 0.25 µg/mL of ethidium bromide added only to the agarose gel is sufficient to detect DNA with comparable sensitivity to the standard method. Adding ethidium bromide to the running buffer is therefore

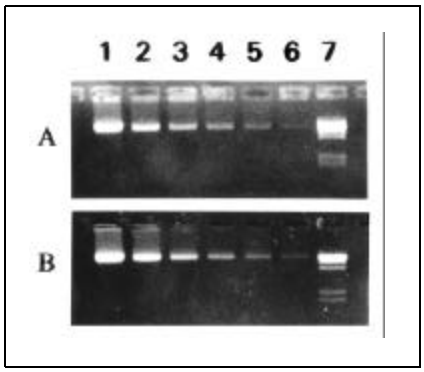

Figure 1. Effect of ethidium bromide in running buffer on DNA staining in agarose gels. Gel A contained 0.5 µg/mL ethidium bromide in both the running buffer and the gel. Gel B contained 0.25 µg/mL ethidium bromide in the gel only. Lane 1: 500 ng; lane 2: 250 ng; lane 3: 125 ng; lane 4: 63 ng; lane 5: 32 ng; lane 6: 16 ng; lane 7: *Hin*dIII-digested λ DNA.

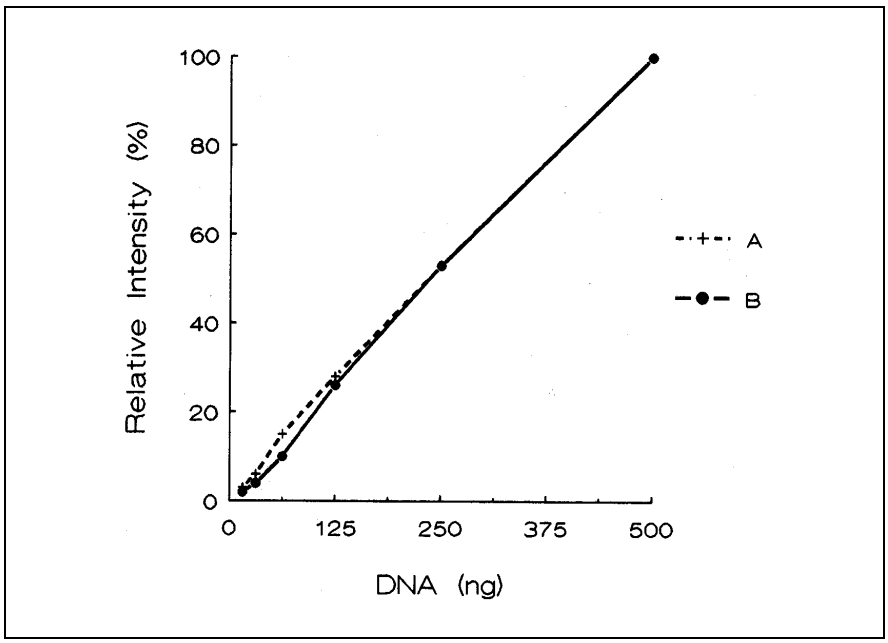

Figure 2. Densitometric analysis of agarose gels run with and without ethidium bromide in the running buffer.

probably unnecessary for most applications, serving only to increase the level of background fluorescence. Omission of ethidium bromide from the running buffer both reduces exposure to this hazardous chemical and markedly reduces the need for time-consuming deactivation procedures (2,3,5).

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This work was supported by a grant from the Liverpool John Moores University Research Fund (to F.C.R.M. and S.M.M.). *Address correspondence to Francis C.R. Manning, School of Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool, L3 3AF England, UK. Internet: f.c.manning@livjm.ac.uk*

Received 3 March 1997; accepted 5 May 1997.

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Simplification of Titer Determination for Recombinant Baculovirus by Green Fluorescent Protein Marker

BioTechniques 23:782-786 (November 1997)

Baculoviruses have emerged as popular vectors for the overproduction of recombinant proteins in eukaryotic cells (2,5,6,9–12). The baculovirus expression system has many advantages, such as correct functionality of the foreign proteins (10,11), high expression level for many proteins (6,10), possibility of posttranslational modification (e.g., glycosylation) (2,5), capacity for large DNA insertions (9), capacity for the expression of unspliced genes, simplicity (e.g., expression kits are commercially available), simultaneous expression of multiple genes and no requirement for transformed cells. Also, the system is nonpathogenic to vertebrates and plants (12).

It is very important to know the titer of a recombinant baculovirus stock, expressed in plaque-forming units (pfu)/mL, when preparing new viral stocks or when carrying out infections for protein production and protein analyses. The productivity of the insect cell/baculovirus expression system is sensitive to cell density, viability, nutrient levels and the multiplicity of infection (MOI), which is the ratio of added virus (pfu) to viable cell at the time of infection. Thus, it is important to know both the viable cell concentration in the bioreactor and the concentration of infectious virus particles in the virus stock. With the exact titer of virus and the viable cell number, one can optimize production of the desired proteins. The viable cell number is determined by hemacytometer counting using Trypan Blue staining (12). The titer of a recombinant baculovirus stock can be determined either by plaque assay (7) or by end-point dilution (12). The advantage of the plaque assay is the potential for exact determination of virus titer. However, the disadvantages of this method are that it is difficult to perform and that it requires a long processing time (approximately 1 week). Furthermore, visualizing plaques can be difficult. On the contrary, the end-point dilution method is very simple. However, results from this method are often more difficult to interpret than plaque assays, particularly when titrating recombinant virus stocks. Wild-type virus is easy to detect because of the accumulation of occlusion bodies, but recombinant virus infection can sometimes be difficult to detect because of the lack of occlusion bodies. To distinguish between infected and uninfected cells, a decrease in cell density and an increase in cell size are monitored (with difficulty) by ordinary light microscopy. To overcome this problem, the β-galactosidase (β-gal) gene (*lacZ*) was introduced into the transfer vectors (16). Therefore, recently, many commercially available transfer vectors contain this infection marker gene. However, the use of this marker requires an additional expensive substrate (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside [X-gal]), which must be added in an additional step near the end of the assay.

By incorporating the gene for the green fluorescent protein (GFP) instead of *lacZ* into the baculovirus, a marker can be created for visualizing gene expression with no need for substrate or other cofactors. GFP was originally

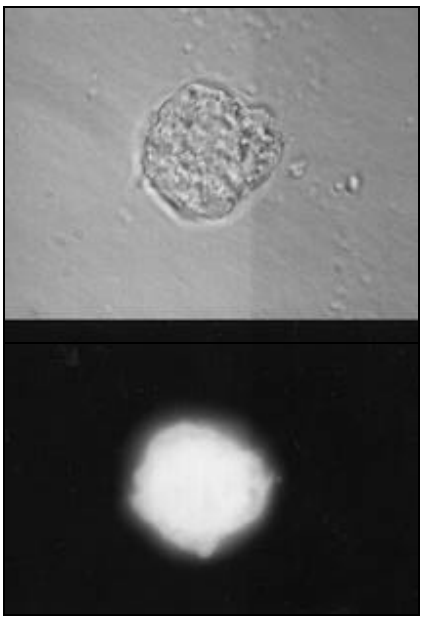

Figure 1. Green fluorescent light emitting Sf9 cells (bottom) and cells under normal light (top). Sf9 cells were photographed using fluorescence microscopy (1000× magnification).