Selective staining with two fluorochromes of DNA fragments on gels depending on their AT content

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Ethidium bromide (EB) is widely used as a fluorochrome for the visualization of DNA on gels. However, the intensity of fluorescence of certain kinds of DNA is relatively low under standard conditions. For example, a given amount of mtDNA from yeast (*Saccharomyces cerevisiae*), which has an extremely high AT content (83%) (1), emits lower fluorescence than the same amount of λDNA. Several dyes are known to bind preferentially to AT-rich regions of DNA (2, 3), but their application to electrophoresis of nucleic acids has not been described, with the exception of one report of the selective staining of DNA in the presence of RNA (4). The present report describes a method for the selective staining of AT-rich fragments of DNA on gels by use of two fluorochromes. Staining with 4′-6-diamidino-2-phenylindole·2HCl (DAPI) (2) in addition to EB makes it possible to visualize AT-rich fragments of DNA as bluish-white fluorescent bands, which are easily differentiated from the red-orange fluorescent bands stained with EB. Two staining procedures can be used, as follows.

Procedure A. The gel is run in the presence of both dyes. DAPI and EB together are incorporated into the gel and the running buffer, prior to electrophoresis, at concentrations of 0.1 and 0.5 μg/ml, respectively. This procedure is convenient when immediate examination of fragments is required, but it is not suitable for the exact determination of sizes of DNA fragments, since AT-rich fragments of DNA stained with DAPI as well as with EB migrate further than expected from their molecular weights. If the gel is run in the presence of EB only, the gel can be stained, after electrophoresis, with DAPI by soaking it in running buffer that contains both DAPI (0.2 μg/ml) and EB (0.5 μg/ml) for 20 min with shaking.

Procedure B. The gel is run in the absence of both dyes. After electrophoresis, the gel is stained by soaking in running buffer supplemented with both DAPI (0.2 μg/ml) and EB (1.0 μg/ml) for 20 min with shaking. After staining, the gel is briefly rinsed, three times, with distilled water. This procedure can be used not only for agarose but also for polyacrylamide gels.

It should be mentioned that DAPI has been reported to require at least three successive AT base pairs as a binding site (5). Therefore, even if DNA fragments have a relatively high AT content on average, they are not always stained with DAPI. However, in the case of a DNA fragment (644 bp) containing the yeast centromere (*CEN3*), which has an AT content of 71% on average, staining with DAPI was observed (data not shown), since the centromere contains AT clusters. If Hoechst 33258 is used instead of DAPI, the bluish-white fluorescence is not as clear as that obtained with DAPI.

Figure 1 shows selective staining by procedure A of several restriction fragments of λDNA, yeast mtDNA and a plasmid that carries a fragment of yeast mtDNA. The λDNA fragments (with an AT content of 51%, lanes 1, 2 and 7) were not stained with DAPI, but exhibited red-orange fluorescence as a result of staining with EB, whereas mtDNA fragments (lanes 3, 4 and 5) showed brilliant bluish-white fluorescence as a result of staining with DAPI. Several fragments of mtDNA exhibited pink fluorescence, intermediate between the bluish-white color due to DAPI and the red-orange due to EB (for example, the first and the fifth bands from the top in lane 3, the fourth band in lane 4). This coloration indicates that these fragments were not extremely rich in AT base pairs. Lane 6 is an example of the application of this selective staining method to a cloning experiment. The third largest *Hin*fI fragment of mtDNA was cloned in pBR322, and only the cloned mtDNA fragment exhibited bluish-white fluorescence due to staining with DAPI.

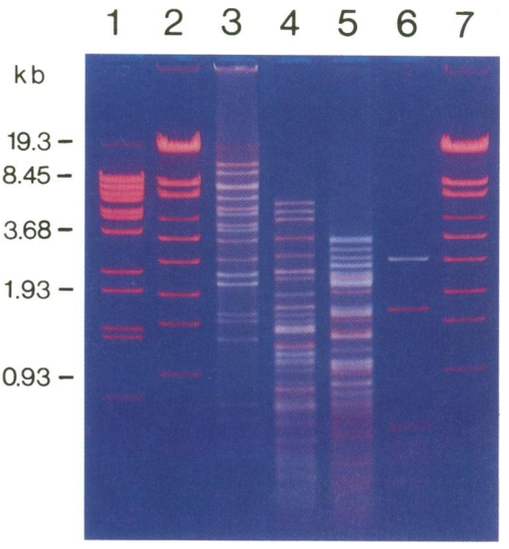

Figure 1. Selective staining of DNA fragments with DAPI and EB on a 1% agarose gel. Lane (1) λDNA digested with *Bst*PI (1.0 μg), (2) λDNA with *Eco*T14I (1.0 μg), (3) yeast mtDNA with *Sst*II (1.5 μg), (4) yeast mtDNA with *Hae*III (1.5 μg), (5) yeast mtDNA with *Hin*fI (1.5 μg), (6) plasmid pHF-1 with *Hin*fI (0.14 μg), (7) DNA with *Eco*T14I (1.0 μg). For irradiation by an overhead UV lamp for the purpose of taking this photograph, 0.14–1.5 μg of DNAs were used. If a transilluminator is used, 0.07–0.75 μg of DNAs are sufficient for detection.

Thus, using our selective staining method, one can easily identify the cloned fragment without hybridization experiments, when an extremely AT-rich sequence is cloned.

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