TECHNICAL NOTE

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The Use of DAPI As a Replacement for Ethidium Bromide in Forensic DNA Analysis

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ABSTRACT: Ethidium bromide is used extensively for the visualization of DNA in forensic applications. This is usually accomplished by the addition of dye to the gel and buffer before electrophoresis or by staining the gel after the run. In both procedures considerable ethidium bromide containing buffer is generated that must be decontaminated. We demonstrate that another fluorescent DNA binding dye, 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI), can be used in place of ethidium bromide for yield and restriction gels, and to stain the visual marker of analytical gels. A low concentration of DAPI is added directly to the DNA. Most of the stain is immediately bound to the DNA and remains bound during electrophoresis.

KEYWORDS: criminalistics, forensic science, DNA, forensic DNA analysis, 4′,6-diamidino-2-phenylindole dihydrochloride, DAPI, DNA visualization, ethidium bromide replacement

DNA based technologies are becoming common techniques in forensic laboratories. These DNA techniques can include the analysis of DNA using restriction fragment length polymorphism (RFLP), polymerase chain reaction (PCR), or wild game identification through satellite DNA comparison. Most applications of these different DNA techniques have a common reliance on ethidium bromide staining to visualize the DNA.

The RFLP protocol developed by the FBI uses ethidium bromide at three different steps of the process to visualize DNA. Routinely two mini gels and one analytical gel are used in this protocol. All of these gels include ethidium bromide in the agarose gel itself and in the electrophoresis tank buffer at a concentration of 0.5 μg/mL resulting in a large volume of contaminated buffer. Ethidium bromide is a potent mutagen and moderately toxic, therefore the buffer generated during the RFLP process must be decontaminated before being discarded by one of several methods [*1*]. Also the use and handling of ethidium bromide solutions and equipment should be done with adequate precautions.

Until recently there were few alternatives to ethidium bromide for staining DNA in agarose gels. Recently a number of new DNA dyes have been reported which extend the limits of detection of DNA in gels [*2–4*]. These are relatively expensive and require complex detection systems [*4*]. None of these are currently used in forensic applications.

We have recently described an alternative to ethidium bromide for use in agarose gels [*5*]. The alternative is the compound 4′,6-diamidino-2-phenylindole (DAPI). This dye binds primarily to AT rich regions in the minor grove of DNA [*6*] yielding a complex that produces an intense bluish-white fluorescence with an emission frequency of 450 nm at an excitation of 365 nm [*7*]. We describe the application of this alternative dye to DNA analysis by RFLP.

Materials and Methods

DAPI (Sigma) was dissolved in deionized water to a stock concentration of 1 mg/mL. The dye solution was added directly to the DNA sample prior to electrophoresis. This can be accomplished by including it in the loading buffer, at a concentration of 10 ng/μL, and adding it to the sample just before electrophoresis. This dye-buffer combination is very stable and has a shelf life of several months at 4°C. The dye solution was not added to the agarose gel or to the electrophoresis tank buffer. The FBI standard protocol was used [*8,9*] with the one change that ethidium bromide was not used in any step in the procedure. DNA fragment lengths were estimated using the FBI sizing program [*10*] using the Lifetechnology/BRL 30 band molecular weight size marker.

Results

An initial experiment was undertaken to determine the ratio of DAPI to DNA required to saturate DNA with the dye. This involved titrating a given amount of lambda DNA (100 ng) with increasing amounts of DAPI holding the volume constant at 6 μL. The dye concentration ranged from 0.1 ng to 3.0 ng. As expected the increase in dye resulted in an increase in fluorescence of the DNA and reached a plateau at a ratio of 0.02 ng DAPI/ ng DNA (results not shown). Using this ratio, the appropriate amount of DAPI could be determined for use in electrophoretic applications required in the RFLP protocol.

Yield and restriction test gels were prepared without ethidium bromide and DNA samples were prepared using loading buffer containing DAPI (10 ng per μL). Two microliters of loading buffer were added to 4 μL of extracted high molecular weight DNA for

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yield gel evaluation. As Fig. 1 indicates, when DAPI concentration is held constant at a suitable DNA to dye ratio, the fluorescence emitted during UV irradiation is correlated with the concentration of DNA present in the sample. This allows easy estimation of sample DNA concentration by comparison to a range of concentration standards run on the same gel. A similar experiment was performed using HAE III restricted DNA samples. The resulting agarose gel containing the restricted DNA is presented in Fig. 2. When DNA concentrations are well above the amounts normally used in yield and restriction gel analysis, the optimum DAPI/DNA ratio is exceeded and the overall fluorescence is no longer related to concentration.

The use of a visual marker in analytical gels to determine the end point of an electrophoretic run is highly recommended [*11*] and can be achieved by monitoring the migration of a known fragmented DNA. This marker is typically observed by either including ethidium bromide in the running buffer and gel or by staining the gel afterwards with ethidium bromide. Figure 3 demonstrates how a visual marker can be easily seen after an analytical electrophoretic run without the use of ethidium bromide. The visual marker used in this instance is a mixture of cut SP6 (Promega) and Ø×174 HAE III fragments stained with DAPI prior to electrophoresis by the inclusion of DAPI in the loading buffer. Experiments in which DAPI was included in either the molecular weight marker or with human DNA on analytical gels show that DAPI slows the migration of DNA, Fig. 4. For this reason we only stain the visual marker with a minimal amount of DAPI and leave the remaining lanes unstained. A comparison of K562 fragment length values, from gels run with or without DAPI included in the visual marker preparation, indicate there is no detectable alteration in the migration of the non-stained DNA. Comparable K562 values were obtained from analytical gels regardless of the inclusion of DAPI with the visual marker (See Table 1). The small amount of retardation that results in the migration of the visual marker is not sufficient to alter its usefulness. DAPI did not interfere in the transfer of DNA to a nylon membrane and subsequent probing as seen by comparison of equal quantities of stained (lane 7) and unstained

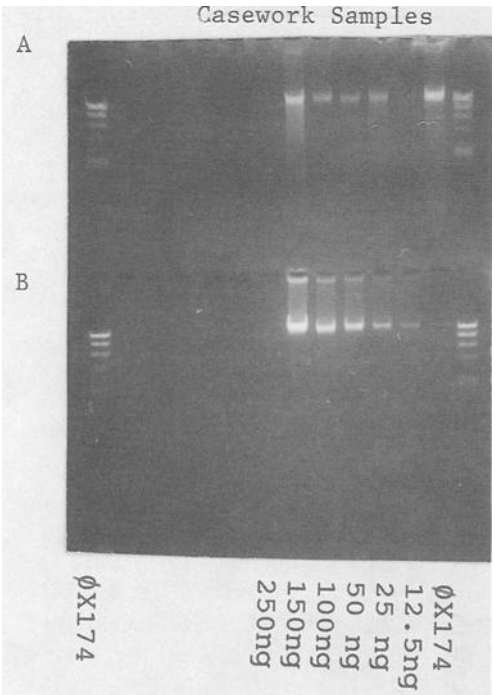

FIG. 1—*A two origin 1% agarose yield gel (6.0 cm × 8 cm) in 1× TAE buffer with DAPI contained only in sample lanes as described in the text. Origin "A" contains typical casework sample extracts and origin "B" contains a dilution of high molecular weight K562 (Promega) to yield the following concentration standards in nanograms loaded per well: 12.5, 25, 50, 100, 150, and 250. Both origin A and B have Hae III cut Ø×174 in outside lanes.*

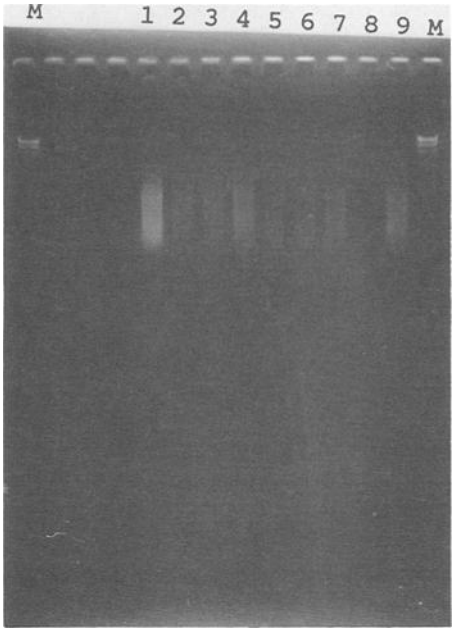

FIG. 2—*Restriction test gel (single origin version of gel described in Fig. 1). Lanes 1–3 are comparison concentration standards with 125, 50, and 30 nanograms loaded per well respectively. Lanes 4–9 are Hae III restricted casework samples, lanes marked "M" contain size marker.*

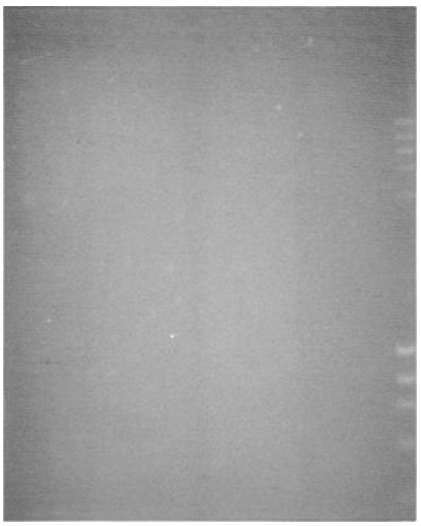

FIG. 3—*Analytical gel, 1% Low EEO agarose (10 × 14 cm) in 1× TAE buffer. The visual marker, a SP6 (1.5 μg) and Hae III Ø×174 (0.3 μg) mix, is stained with 40 ng of DAPI. All other lanes are without DAPI.*

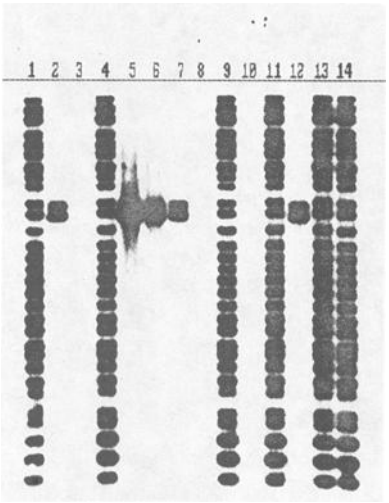

FIG. 4—*Video image of an autoradiogram of an analytical gel. Ten nanograms of DAPI were added to various concentrations of K562 and to selected molecular weight marker lanes. Lanes 5–8 contain DAPI and 2, 1, 0.4 and 0.05 micrograms of K562 DNA respectively. Lanes 2 and 12 contain 0.4 micrograms of unstained K562. The BRL 30 band size marker in lanes 11 and 13 also contain DAPI. All other lanes are not DAPI stained. The resulting membrane was subjected to three probings (D2S44, D1S7, & D10S28) with D1S7 shown. The DAPI stained K562 DNA migrated somewhat more slowly than non-stained DNA in all probings, resulting in an apparent 0.6% larger molecular weight band size on average. Unstained K562 DNA evaluated with DAPI stained molecular weight markers yielded values on average 1.1% smaller than the same K562 band evaluated with non-stained markers.*

TABLE 1—*K562 cell line fragment length values in base pairs from analytical gels with or without DAPI-stained visual marker.*

	Loci examined			
	D4S139	D1S7	D2S44	D10S28
Unstained gels	6543	4599	2914	1764
	3455	4245	1797	1189
DAPI-Stained	6510	4614	2912	1765
Visual Marker	3454	4253	1793	1188

NOTE: Data for each locus is the average of the cell line control data from eight separate gels.

The K562 measured in each gel was adjacent to the first molecular weight ladder lane nearest the DAPI-containing visual marker lane.

(lanes 2 and 12) K562 and comparison of stained (lanes 11 and 13) and unstained (lanes 1,4,9, and 14) ladder in Fig. 4.

Discussion

The use of DAPI allows one to visualize DNA in the concentration range required in forensics and it can replace the ethidium bromide currently used in every step of the typical RFLP DNA analysis. DAPI has a similar sensitivity to that of ethidium bromide and can easily replace it in yield, restriction and analytical gels. In our hands ethidium bromide still gives slightly better DNA detection at very low concentrations and if desired, one always has the option to place the DAPI stained gel in ethidium bromide. The resulting double stained gel can be easily photographed using standard methods for ethidium bromide photography. Routine case work demands usually do not require this additional step. We have tried to use ethidium bromide in the same fashion as DAPI is used in this report. However, when ethidium bromide is included only in the loading buffer, it does not remain bound to DNA during electrophoresis, and instead migrates towards the cathode [*1*].

DAPI is also very easy to use and stable when frozen. When DAPI is included in the loading buffer, there are no additional steps required to perform a yield or restriction gel analysis. We have also found that the known concentration standards used in yield gel analysis can be prepared with DAPI and used for months if kept frozen between uses. Our DAPI stock solution has been in use for over two years with no detectable loss in staining capacity. Many DNA dyes suffer from light degradation and must be shielded from the light. Although we are careful in limiting the time DAPI solutions are kept out at room temperature and in light, we have never realized a need to work in the dark or in haste.

The photography of DAPI stained gels is similar to that used with ethidium bromide. The same equipment that is used for routine ethidium photography is also used with DAPI except that the filters required are different. We have found that the combination of Wratten #2 and #3 are suitable for most situations. The excitation wavelength for DAPI is close to the emission wavelength and so the choice of filters required to record the fluorescence complex is very important. We have also found that many UV transparent gel trays designed for use in UV photography are not suitable in conjunction with DAPI since these trays produce background radiation similar to the emission wavelength of DAPI. To overcome this problem we routinely place gels on plastic wrap before placing them on the UV light box.

Only a few studies have been conducted on the health effects of DAPI [*12,13*]. However, limiting one's exposure by greatly reducing the amount of DNA dye required for RFLP analysis should substantially reduce the potential for exposure to harmful substances.

References

[*1*] Sambrook, J., Fritsch, E. F., and Maniatis, T., "Commonly Used Techniques in Molecular Cloning," In *Molecular Cloning: A Laboratory Manual*, N., Irwin, N., Ford, C., Nolan, and M., Ferguson, Eds., Cold Spring Harbor University Press, Cold Spring Harbor, NY, 1989.

[*2*] Glazer, A. N., Peck, K., and Mathies, R. A., "A Stable Double-Stranded DNA-Ethidium Homodimer Complex: Application to Picogram Fluorescence Detection of DNA in Agarose Gels," *Proceedings of the National Academy of Sciences USA*, Vol. 87, 1990, pp. 3851–3855.

[*3*] Rye, H. S., Quesada, M. A., Peck, K., Mathies, R. A. and Glazer, A. N., "High-Sensitivity Two-Color Detection of Double-Stranded DNA with a Confocal Fluorescence Gel Scanner Using Ethidium Homodimer and Thiazole Orange," *Nucleic Acids Research*, Vol. 19, 1990, pp. 327–333.

[*4*] Rye, H. S., Yue, S., Wemmer, D. E., Quesada, M. A., Haugland, R. P., Mathies, R. A., and Glazer, A. N., "Stable Fluorescent Complexes of Double-Stranded DNA with Bis-Intercalating Asymetric Cyanine Dyes: Properties and Applications," *Nucleic Acids Research*, Vol. 20, 1992, pp. 2803–2812.

[*5*] Buel, E. and Schwartz, M. B., "DAPI, a Simple Sensitive Alternative to Ethidium Bromide Staining of DNA in Agarose Gels," *Applied and Theoretical Electrophoresis*, Vol. 3, 1993, pp. 253–255.

[*6*] Wilson, W. D., Tanious, F. A., Barton, H. J., Jones, R. L., Fox, K., Wydra, R. L., and Strekowski, L., "DNA Sequence Dependent Binding Modes of 4′,6-diamidino-2-phenylindole (DAPI)," *Biochemistry*, Vol. 29, 1990, pp. 8452–8461.

[*7*] Williamson, D. H. and Fennel, D. J., "The Use of Fluorescent DNA-Binding Agent for Detecting the Separation of Yeast Mitochondrial

DNA," In Methods in Cell Biology, D. M. Prescott, Ed., Vol. 12, 1975, Academic Press, NY, pp. 335–351.

[8] Budowle, B. and Baechtel, F. S., "Modifications to Improve the Effectiveness of Restriction Fragment Length Polymorphism Typing," *Applied and Theoretical Electrophoresis*, Vol. 1, 1990, pp. 181–187.

[9] Budowle, B., Waye, J. S., Shutler, G. G., and Baechtel, F. S., "Hae III-A Suitable Restriction Endonuclease for Restriction Fragment Length Polymorphism Analysis of Biological Evidence Samples," *Journal of Forensic Sciences*, Vol. 35, No. 2, 1992, pp. 404–424.

[10] Monson, K. L., "Semiautomated Analysis of DNA Autoradiograms," *Crime Laboratory Digest*, Vol. 25, 1988, pp. 104–105.

[11] Technical Working Group on DNA Analysis Methods (TWGDAM), "Guidelines for a Quality Assurance Program for DNA Analysis," *Crime Laboratory Digest*, Vol. 18, No. 2, April 1991, pp. 44–75.

[12] Park, C. H., Kimler, B. F. and Smith, T. K., "Comparison of the Supravital DNA Dyes Hoechst 33342 and DAPI for Flow Cytometry and Clonogenicity Studies of Human Leukemic Marrow Cells," *Experimental Hematology*, Vol. 13, 1985, pp. 1039–1043.

[13] Durand, R. E. and Olive, P. L., "Cytotoxicity, Mutagenicity and DNA Damage by Hoechst 33342," *Journal of Histochemistry and Cytochemistry*, Vol. 30, 1982, pp. 3851–3855.

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