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Cyanine dyes for the detection of double stranded DNA

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Abstract

Twenty three novel cyanine dyes have been applied as fluorescent stains for the detection of nucleic acids in agarose gel electrophoresis. Significant fluorescence enhancement of these dyes in the presence of double stranded DNA was observed. Five dyes offered superior sensitivity in the detection and quantification of DNA, over Ethidium Bromide, the most commonly used nucleic acid stain.
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1. Introduction

The enhanced fluorescence of Ethidium Bromide upon binding to nucleic acids, which had been observed by LePecq and Paoletti [1], was first exploited for the detection of DNA in electrophoresis gels by Sharp, Sugden and Sambrook in 1973 [2]. Since then this technique has become one of the most widely used in molecular biology [3].

However, the use of Ethidium Bromide is not without drawbacks, it is fluorescent in its unbound state which decreases the signal-to-background ratio and thus diminishes the ability to detect very small amounts of nucleic acids [4]. Additionally, it is a powerful mutagen [5], moderately toxic [6] and must be disposed of safely [7,8].

Recently the cyanine dyes have found use in a large number of diverse fields. They are used extensively in biological, medical and drug development areas as fluorescent labels and probes [9–23] for cells, micelles and organelles [24–27], detection of proteins [28,29], conformational studies via fluorescence energy transfer [30–32], flow cytometry [33–35], fluorescence microscopy [36], DNA sequencing [37,38], detection on microarrays [39], quantification of nucleic acids in capillary and gel electrophoresis [40–42] and single molecule detection [43]. The development of automated, high-throughput screening (HTS) of drug candidates produced by combinatorial synthetic methods has been a significant new outlet for fluorescent probes and reagents [44,45].

Many cyanine dyes offer improved sensitivity over Ethidium Bromide in the detection and quantification of nucleic acids in solutions and gels owing to their very low intrinsic fluorescence while exhibiting a large (often over 1000 fold) enhancement upon binding to nucleic acids [9].

In this study 23 cyanine dyes have been applied to agarose gel for the fluorescent detection and quantification of DNA fragments. Ethidium Bromide and TOTO [1,1′-(4,4,7,7-tetramethyl-4,7-diazaundecamethylene)-bis-4-[3-methyl-2,3-dihydro-(benzo-1,3-thiazole)-2-methyl-idene]-quinolinium tetraiodide] was also included as a standard.

2. Materials and methods

2.1. Dyes

Cyanine dyes were from Avecia. The structures of the dyes are presented in Table 1. Stock solutions of 20 mg/ml were made in DMSO and were diluted in distilled water to a concentration of 1 μg/ml immediately prior to use.

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Table 1
Dye structures

Name	λ_{max}	Structure
Dye 1	215, 525	
Dye 2	215, 525	
Dye 3	215, 560	
Dye 4	215, 536	
Dye 5	215, 526	
Dye 6	215, 508	
Dye 7	215, 516	
Dye 8	215, 528	
Dye 9	215, 522	
Dye 10	215, 524	
Dye 11	215, 544	
Dye 12	215, 508	
Dye 13	215, 496	
Dye 14	215, 482	
Dye 15	215, 540	
Dye 16	215, 488	
Dye 17	215, 498	
Dye 18	215, 540	
Dye 19	215, 554	
Dye 20	215, 554	
Dye 21	215, 556	
Dye 22	215, 504	
Dye 23 Ethidium Bromide	300, 495	
Dye 24	215, 508	
Dye 25	215, 514	

2.2. Dna

λ Hind III Digest DNA was obtained from SIGMA. Lambda is a linear DNA isolated from *E. coli* bacteriophage containing 48,502 base pairs, which has been digested by a restriction enzyme, *Hind* III, to give seven fragments as shown in Fig. 1.

Lambda phage DNA contains 10–16 base single-stranded regions at the 5' and 3' termini that are self complimentary, called cos ends. For example the 4361 and 23,130 base pair fragments will hybridise at these cos sites, leading to a decrease in the amount of 4361 base pair fragment. Therefore, in order to properly resolve lambda phage DNA fragments the DNA was heated to 65 °C to melt any aggregation due to these 'sticky ends'.

200 ng of DNA was deposited to each gel well prior to electrophoresis.

2.3. Electrophoresis of DNA in agarose gel

Electrophoresis of DNA in 0.75% agarose was carried out by standard methods [46] in 0.1X TBE buffer (0.09 M Tris base; 0.09 M boric acid; 2 mM EDTA; pH 8.0). The gel was run for 2 h at 10 V/cm.

2.4. Staining of DNA and gel photography

The visualisation of DNA in electrophoretic gels can be achieved either by pre-staining, in which an aliquot of dye is added to the agarose solution prior to casting, or post-staining the gel in a bath after electrophoresis. The decision as to whether pre- or post-staining is appropriate is determined by the dissociation constant of the dye-DNA complex. If the dissociation constant is greater than the magnitude of the electric field applied during electrophoresis then the complex will be stable and pre-staining is possible. Ethidium Bromide can be used as both pre- and post-stain. Pre-staining provides a more simple approach, requiring one less step, whereas post-staining is considered to provide greater sensitivity in detecting DNA [47].

The dissociation constants of the dye-DNA complex of dyes assessed in this study were not known. Therefore post-staining was conducted.

After electrophoresis the gel was cut into strips, each containing one lane of equal quantity of λDNA. Each strip was stained in freshly prepared solution of one of the cyanine dyes for 1 h, followed by a rinse in distilled water. Each strip was then examined on a UV-transilluminator. Agarose gels were photographed using a Syngene Geneflash and images were analysed by Total Lab TL120 from Nonlinear Dynamics [48]. Orange (for Ethidium Bromide) and green filter (Cyanine dyes) were used during photography.

Reproducibility was shown to be good by repeating the gel experiment using Dye1 10 times. The fluorescence of the 95 ng band was measured each time. The results of which gave a mean = 106.3 and a standard deviation = 1.27.

3. Results

The 23 cyanine dyes were examined for their ability to visualise DNA in agarose. The results are presented in Table 2.

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