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New visible and selective DNA staining method in gels with tetrazolium salts

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ABSTRACT

DNA staining in gels has historically been carried out using silver staining and fluorescent dyes like ethidium bromide and SYBR Green I (SGI). Using fluorescent dyes allows recovery of the analyte, but requires instruments such as a transilluminator or fluorimeter to visualize the DNA. Here we described a new and simple method that allows DNA visualization to the naked eye by generating a colored precipitate. It works by soaking the acrylamide or agarose DNA gel in SGI and nitro blue tetrazolium (NBT) solution that, when exposed to sunlight, produces a purple insoluble formazan precipitate that remains in the gel after exposure to light. A calibration curve made with a DNA standard established a detection limit of approximately 180 pg/band at 500 bp. Selectivity of this assay was determined using different biomolecules, demonstrating a high selectivity for DNA. Integrity and functionality of the DNA recovered from gels was determined by enzymatic cutting with a restriction enzyme and by transforming competent cells after the different staining methods, respectively. Our method showed the best performance among the dyes employed. Based on its specificity, low cost and its adequacy for field work, this new methodology has enormous potential benefits to research and industry.

1. Introduction

The separation and visualization of DNA by electrophoresis is associated with a variety of analytical and diagnostic assays. Visualization is carried out through different methods such as silver staining and various fluorescent dyes like ethidium bromide (EB) and SYBR Green I (SGI) [1,2]. Although silver staining is very sensitive, it lacks selectivity and specificity, does not allow sample recovery and usually takes several hours. On the other hand, the use of fluorescent dyes allows analyte recovery and is more selective than silver staining, but requires instruments such as a transilluminator (UV or blue light) or fluorimeter for visualization [3]. Further, dyes such as EB require UV irradiation, which causes damage to both the DNA sample and the experimentalist [4], and uses visualization equipment. Finally, DNA labeling with radioactive isotopes is very sensitive, but is both hazardous and expensive, limiting its application in most laboratories [5,6].

There are a few visible DNA staining methods, which use dyes such as methylene blue, brilliant cresyl blue [7], crystal violet [8], Nile blue [9,10] and ethyl violet [11]. However, although safe, these methods require long staining times, lack sensitivity and are not specific for nucleic acids.

In other scientific fields (e.g., cell metabolism and viability measurements, histochemistry assays and alkaline phosphatase assays), visualization has been accomplished by means of tetrazolium salts, which are organic oxidants that are in themselves colorless or weakly colored. The principle of those methods is the reduction of tetrazolium salts to form a brightly colored formazan product mainly through NADH and NADPH action [12–15]. Of interest to the nucleic acid field, bivalent tetrazolium salts with electron withdrawing chemical groups, like Nitro Blue Tetrazolium

Abbreviations: NBT, nitro blue tetrazolium; SGI, SYBR Green I; SGII, SYBR Green II; EB, Ethidium bromide; GR, gel red; SG, SYBR gold.

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(NBT), have been shown to bind DNA using their positive tetrazolium rings [16].

Here we describe a novel visual and highly selective DNA staining method, using a combination of NBT, a fluorescent dye (which binds the DNA), and sunlight, to produce a purple precipitate that remains in the gel after exposure to light. This method improves DNA integrity after recovery from gels in comparison with UV-based methods. Also, it does not require UV light or instruments to visualize the bands during the daytime, although the reaction can be triggered just by exposure to blue light.

2. Materials and methods

2.1. Materials

The compounds used were obtained from the following companies: SYBR stains, PageRuler unstained protein ladder, GeneRuler 1 kb plus DNA ladder, acrylamide and XbaI enzyme, from ThermoFisher (Waltham, MA, US); Gel Red from Biotium (Hayward, CA, US); NBT from Gold Biotechnology (St. Louis, MO, US); bisacrylamide from SIGMA (St. Louis, MO, US); ssRNA ladder from New England Biolabs (Ipswich, MA, US); Gel Extraction Kit from QIAGEN (Hilden, Germany); Bacto™ Yeast Extract from Becton, Dickinson and Company (Sparks, MD US); lipopolysaccharides (LPS) extract was kindly donated by Dr. Sergio Alvarez, Universidad de Chile. All other reagents were obtained from Merck (Darmstadt, Germany).

2.2. Staining methods: fluorescent and visible staining

The fluorescent dyes GelRed (GR), SYBR Gold (SG), SGI and SYBR Green II (SGII), were dissolved in distilled water at concentrations recommended by the manufacturers and the gels were immediately soaked in these solutions with agitation and then digitalized in SYNGENE G:BOX Chemi XT4, Synoptics Ltd (Nuffield Road, Cambridge, UK). Acrylamide gels were stained for 10 min; agarose gels for 1 h. EB staining was carried out as described by Brunk and Simpson (1977) [16]. Coomassie staining was carried out as in Green and Sambrook (2012) [18]. Silver staining was carried out as in Martinić et al. (2011) [17].

2.3. NBT-dye method

For the new combined NBT-dye staining method, the fluorescent dye was diluted in 82.25% of the final volume and enough NBT 0.1% (w/v) was added so the final concentrations were 2 μM and 0.25 mM NBT. The gel was then soaked in the solution (with stirring) for 25 min in the dark, followed by exposure to sunlight for 90 min. Sunlight intensity (470 nm) was measured with a Power Meter Console PM100A, Thorlabs (Newton, NJ, US) set up with Photodiode Power Sensor S130C, Thorlabs (Newton, NJ, US). A schematic representation of the method and a representative gel are shown in Supplemental Fig. 1 and Fig. 1, respectively.

2.4. Digitalization methods

Fluorescence gels were digitalized using SYNGENE G:BOX Chemi XT4 using UV illumination. For NBT, Coomassie and silver staining, the polyacrylamide gels were covered between transparent plastic sheets; the gels were scanned using Epson L210, Epson (Suwa, Nagano, Japan) scanner at 1200 gray scale and color configuration. Photoshop CS6 (San Jose, CA, US) was used for image straightening. Image analyses were carried out using UN-SCAN-IT (Orem, UT, US). Statistical analyses were carried out using Sigmaplot 12.5 (San Jose, CA, US).

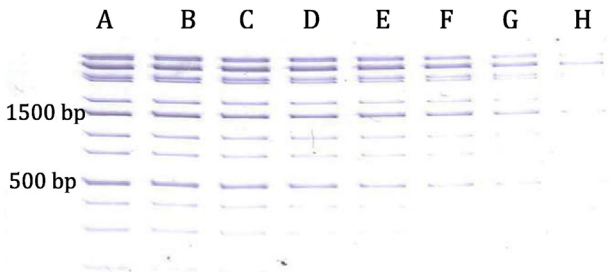

Fig. 1. Calibration curve of NBT-SGI. Scanned 12.5% non-denaturing polyacrylamide gel loaded with 66.6 to 3.3 ng of DNA ladder. After electrophoresis, the gel was stained with NBT-SGI and rinsed thrice with distilled water. The wells were loaded with a DNA ladder which had the following amounts of DNA (ng) in the 1500-bp, and 500-bp bands (respectively): A) 10.7, 10.0; B) 8.5, 8; C) 6.4, 6; D) 4.7, 4.4; E) 3.5, 3.3; F) 2.3, 2.2; G) 1.2, 1.1; H) 0.53, 0.50.

2.5. Calibration curves

A calibration curve between 4 and 125 ng of DNA was obtained by loading a DNA ladder in non-denaturing 12.5% polyacrylamide gels and electrophoresis was performed at 100 V in TAE 1X for 2 h. The gels were then stained using the NBT-dye method. This procedure was repeated using the following dyes: SGI, SG, GR and EB in combination with NBT. For NBT-EtBr staining, the DNA calibration curve was between 125 and 1250 ng of DNA.

2.6. DNA recovery and integrity comparison

The pDJ100 plasmid [20], kindly provided by the Schekman laboratory (UC Berkeley, US) with an initial amount of 4765 (±100) ng was electrophoresed in a 0.8% agarose gel in TAE 1X at 100 V for 1 h. The different gels were stained with NBT-SGI, SGI, and two unstained gels were used as controls (non-staining with UV (Ctrl UV(+)), and non-staining non UV (Ctrl UV (−)) and the sample's band was extracted with a scalpel. When not stained, the control bands (Ctrl UV(+) and Ctrl UV (−)) were extracted according to their retention factor (R_f). The DNA was then extracted using a QIAGEN gel extraction kit. Finally, the gels were stained using SG and analyzed as mentioned above, using the percentage of cut DNA against total loaded DNA.

The integrity of the DNA samples was measured through an enzymatic cutting of 350 ng of DNA treated with the XbaI restriction enzyme for 1 h at 37 °C. After treatment the samples were loaded in 5% non-denaturing polyacrylamide gels and electrophoresis was performed in TAE 1X at 100 V for 2 h. As negative controls, XbaI non treated DNA was loaded into the gels used. Finally the gels were stained using SG and lately analyzed as mentioned above using the percentage between cut DNA against total loaded DNA. On other hand, transformation of 50 μL of competent *E. coli* XL10 gold cells was performed as in Inoue et al. (1990) [19] with 5 μL of 20 ng/μL of treated plasmid to test DNA functionality after extraction of the different stained DNAs. The transformed cells were grown in selective LB-ampicillin agar medium, and were quantified by the dilution method described in Madigan et al. (2003) [21]. The number of colonies were counted and analyzed.

2.7. Identification of the precipitated electrophoretic band by mass spectrometry

Non-denaturing polyacrylamide gels (12.5%) were loaded with 125 ng of DNA ladder; after electrophoresis, the gels were stained with NBT-SGI and rinsed thrice with distilled water. About 45 DNA stained bands were excised with a scalpel, with the gel slices being