Video Article

DNA Staining Method Based on Formazan Precipitation Induced by Blue Light Exposure

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Abstract

DNA staining methods are very important for biomedical research. We designed a simple method that allows DNA visualization to the naked eye by the formation of a colored precipitate. It works by soaking the acrylamide or agarose DNA gel in a solution of 1x (equivalent to 2.0 µM) SYBR Green I (SG I) and 0.20 mM nitro blue tetrazolium that produces a purple precipitate of formazan when exposed to sunlight or specifically blue light. Also, DNA recovery tests were performed using an ampicillin resistant plasmid in an agarose gel stained with our method. A larger number of colonies was obtained with our method than with traditional staining using SG I with ultraviolet illumination. The described method is fast, specific, and non-toxic for DNA detection, allowing visualization of biomolecules to the "naked eye" without a transilluminator, and is inexpensive and appropriate for field use. For these reasons, our new DNA staining method has potential benefits to both research and industry.

Video Link

The video component of this article can be found at https://www.jove.com/video/56528/

Introduction

With the advances in biochemistry and molecular biology, the studies involving DNA have required better techniques to analyze DNA. The first method for DNA staining was silver staining, which is very sensitive but lacks selectivity and does not allow sample recovery. Later, the development of fluorescent DNA staining allowed the selective quantification of DNA with the possibility of sample recovery. One of the first fluorescent dyes used for DNA quantification was ethidium bromide[1], which is mutagenic[2]. However, now there are improved fluorescent dyes that are safer and more sensitive, such as GelRed and SYBR Green I (SG I)[3]; but all of these fluorescent dyes require the use of an ultraviolet (UV) transilluminator or fluorimeter.

There are other techniques for visibly staining DNA, such as methylene blue[4] and crystal violet[5,6,7,8,9], but all of these suffer from reduced sensitivity and selectivity.The tetrazolium salts are organic compounds susceptible to reduction, and when this happens they form an insoluble and colored formazan precipitate[10,11]. Recently, some bivalent tetrazolium salts have been shown to bind to DNA due to their positive tetrazolium rings[12].

In a recent publication[13], a new visible technique to stain and quantify DNA in polyacrylamide gels was proposed, using the reduction of a bivalent tetrazolium salt called nitro blue tetrazolium (NBT) and fluorescent dyes like ethidium bromide, GelRed, SYBR Green I and SYBR Gold. This reaction worked in the presence of blue light or sunlight and allowed sample recovery with improved quality compared to the use of SG I with a UV transilluminator.The objective of this paper is to provide a detailed protocol of the staining technique using tetrazolium salts.

Protocol

1. Preparing and Running the Gel

1. Prepare the non-denaturing 12% polyacrylamide gel gels using the Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) recipe found in Sambrook and Russell[14] but using water instead of SDS.
    1. To prepare 5 mL of resolving gel, use 1.7 mL of distilled water, 2 mL of acrylamide/bis acrylamide (30/0.8% w/v), 1.3 mL of 1.5 M Tris pH 8.8, 0.05 mL of 10% ammonium persulfate, and 0.002 mL of TEMED.
    2. To prepare 2 mL of stacking gel use 1.5 mL of water, 0.33 mL of acrylamide/bis acrylamide (30/0.8% w/v), 0.25 mL of 1 M Tris pH 6.8, 0.02 mL of 10% ammonium persulfate, and 0.002 mL of TEMED. The dimensions of the gel are 7.3 cm x 8.2 cm x 0.075 cm (l x w x d). **Note:** Use a 15 well comb of 0.75 mm thickness for the best sensitivity.
2. Add the appropriate amounts of 6x loading buffer and DNA sample to a 1.5 mL microcentrifuge tube (*e.g.* use 1 µL of 6x loading buffer to 5 µL of DNA sample).

3. Place the gel in the vertical electrophoresis chamber.
    1. Fill the chamber with 750 mL of 1x TAE buffer to run 2 gels. To prepare 1 L of 50x TAE, use 442 g of Tris base, 57.1 mL of glacial acetic acid, and 100 mL of 0.5 M EDTA pH 8. Add distilled water to a final volume of 1 L.
    2. Load the samples in the gel. Run the gel at 100 V for 2 h.

4. Alternatively, prepare a 1x TAE agarose gel using the recipe found in Sambrook[14].
    1. Add 300 mL of 1x TAE to the horizontal electrophoresis chamber. Run the gel for 45 min at 100 V.

2. NBT-SG I Staining (Figure 1).

1. **Acrylamide Gel Staining.**
    1. Prepare the solutions: 0.1% w/v NBT and 1.2x SG I.
    Note: It is possible to use ethidium bromide, GelRed and SYBR Gold instead of SG I, but SG I had the best performance.
    2. Pour 16.75 mL of 1.2x SYBR Green I solution into a container of appropriate size.
    Note: We used the lid of a pipette tip box as a container, and these volumes are calculated to cover the gel using this container; its dimensions are 11.6 cm x 7.6 cm x 2.6 cm (l x w x d).
    3. Place the gel in the solution. Add 3.25 mL of 0.1% w/v NBT solution to give 0.2 mM NBT and 1x (equivalent to 2.0 µM) SG I final concentrations.
    4. Seal the container with plastic film, and completely wrap the container with aluminum foil to block any ambient light.
    Note: The plastic film is used to prevent splashing and to avoid reaction of the aluminum foil with the staining solution.
    5. Shake for 25 min on an orbital shaker at 60 rpm.
    6. Remove aluminum and plastic cover. Expose to sunlight for 90 min maximum. Check every 5 min until the band of interest appears.
    7. To use blue light, prepare a protoboard with 3 Light-Emitting Diodes (LED) and place the LEDs about 5 mm from the gel surface (check the staining continuously, as the time needed will depend on the intensity of the LED light source).

2. **Agarose Gel Staining.**
    1. After preparing and running a 1% agarose gel as described in sections 1.4 (the dimensions are 1 cm x 6.2 cm x 7 cm; l x w x d), follow the same steps as in 2.1 with these modifications:
    2. Pour 41.9 mL of 1.2x SG I solution into the lid of a pipette tip box; its dimensions are 11.6 cm x 7.6 cm x 2.6 cm (l x w x d). Add 8.1 mL of 0.1% NBT solution.
    3. Seal the container with plastic film, and then completely wrap the container with aluminum foil to block any ambient light.
    4. Shake for 1 h on an orbital shaker at 60 rpm.

3. Data Analysis.

1. **NBT-SG I Calibration Curves.**
    1. Obtain gel images by scanning at 1200 dpi.
    2. Open software for image analysis. Straighten the images using an image editor. Run an image densitometry.
    3. Calculate the area under the curve. Prepare a graph of Signal/$Signal_{Max}$ versus DNA concentration.

4. DNA Recovery.

1. Prepare a 1% agarose gel in 1x TAE using the recipe found in Sambrook[14].
2. Add 300 mL of 1x TAE to the horizontal electrophoresis chamber. Load 4 µL of 100 ng/µL pDJ100[15] plasmid and DNA ladder following the scheme given in **Figure 2**. Run the gel for 1 h at 100 V.
3. Mark and weigh two 2 mL microcentrifuge tubes.
4. Cut the gel down the middle in order to have two identical halves. Each one should contain the ladder and the sample plasmid to compare the different staining techniques. A schematic representation is in **Figure 2**.
5. Stain one half with NBT-SG I as in step 2.
    1. Cut out the plasmid band using a clean scalpel. Save the gel piece in one tube and weigh.

6. Stain the other part of the gel with SG I with 50 mL of 1x SG I solution and slowly shake (60 rpm) the gel for 1 h in darkness.
    1. Place the gel on a transilluminator and expose for 2 min. Cut out the plasmid band using a clean scalpel.
    2. Save the gel piece in the other tube and weigh.
    Note: The protocol can be paused here while keeping the tubes at -20 °C.

7. Carry out plasmid extraction using a commercial gel extraction kit.

5. Plasmid Normalization.

1. In a 1.5 mL microcentrifuge tube, add 10 µL of extracted plasmid and 60 µL of type I water. Mix and transfer to a 60 µL quartz cuvette or use 2 µL directly in a Nanodrop.
2. Measure absorption spectra between 230 to 320 nm. Calculate the sample's plasmid concentration using the following formula:
$$DNA\ concentration\ (\mu g/mL) = (A_{260} - A_{320}) * D * 50$$
where *D* is the dilution factor applied (in this case it is 70/10), A_x is absorption at *x* nm, and 50 is the conversion factor to µg/mL for dsDNA.

3. Dilute samples to 9.1 ng/µL.
4. Transform *Escherichia coli* (*E. coli*) competent cells (e.g. XL10 Gold) following the Inoue transformation procedure[16] using 5 µL of dilute sample.
5. Count the number of colonies on the Petri dishes and note the dilution. Calculate the Colony Forming Units (CFU) with the following formula[17]:

   $CFU = N°colonies\ per\ plate * D$

   where *D* is the dilution factor applied from the initial culture.
6. Perform an unpaired t-test.

Representative Results

A purple formazan precipitate appears where the DNA is located (verified by mass spectrometry[13]). In the experiment, a DNA ladder was loaded to make a calibration curve (**Figure 3**). The protocol works for both acrylamide and agarose gels, but it has a lower intensity and takes a longer time in agarose. However, the use of agarose gels allows the recovery of the sample using a commercially available kit, and it does not interfere with the extraction. The samples recovered using this method with blue LEDs have a better quality compared to SG I using a transilluminator (**Figure 4**).

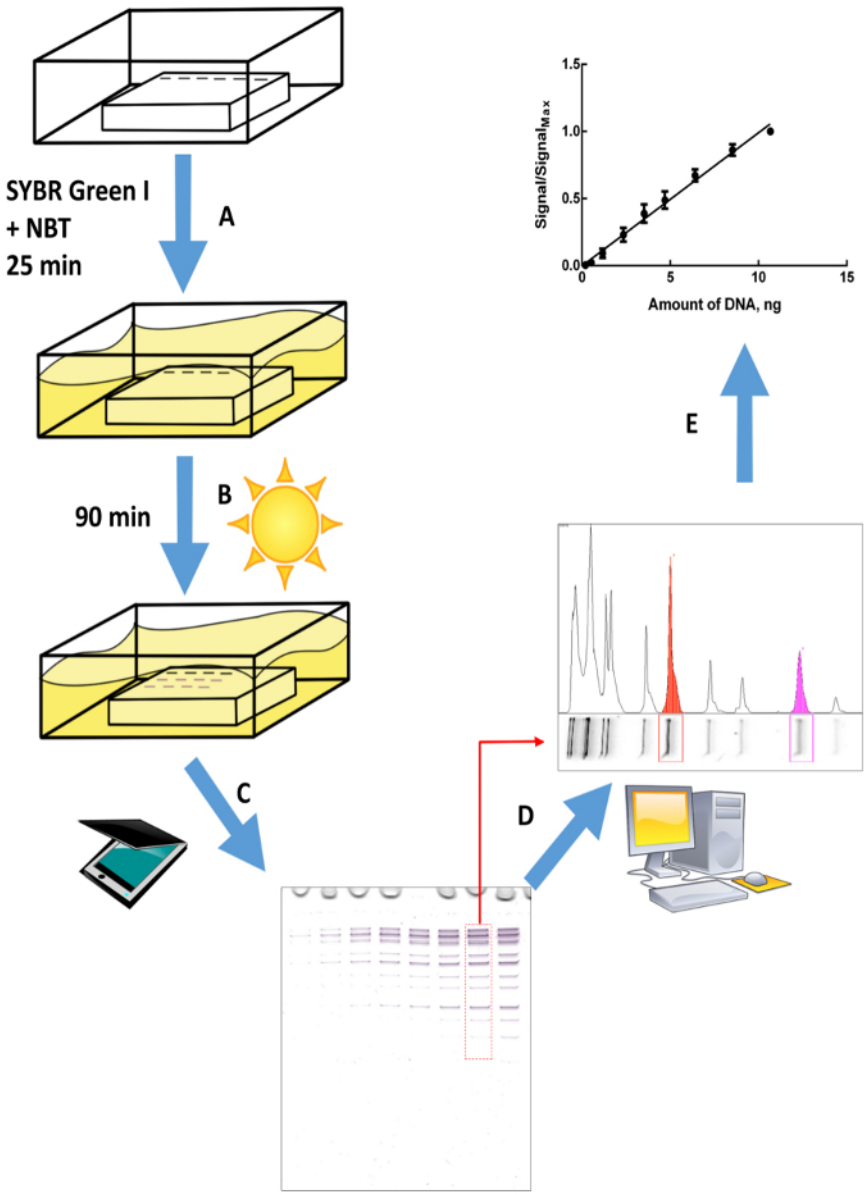

Figure 1.Schematic of NBT-SG I DNA staining and quantification analysis. The steps are: A. Adding SG I and NBT solutions to the gel. B. Exposing the gel to sunlight or blue light. C. Scanning the gel at 1200 dpi. D. Analyzing the gel image by an image processing software to get band intensity. E. Plotting the data in an intensity versus concentration plot. Please click here to view a larger version of this figure.

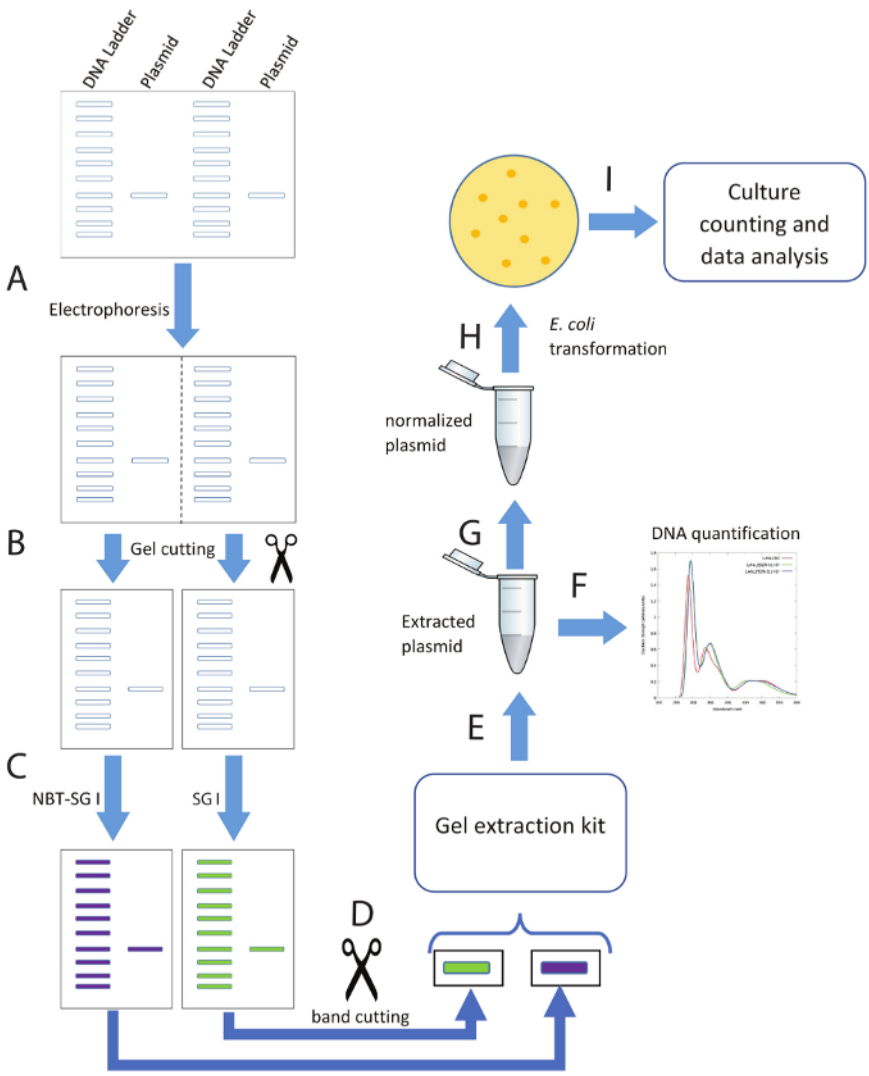

Figure 2. Schematic of DNA recovery and integrity comparison assay. A. DNA electrophoresis. B. Cutting gel into two pieces. C. Staining with the two different staining methods (SG I and NBT-SG I). D. Cutting out plasmid DNA band. E. DNA gel extraction procedure using extraction kit. F. DNA quantification by spectrophotometry measurement. G. DNA concentration normalization to compare the two methods. H. Bacterial transformation with the samples after normalization I. Colony counting and data analysis. Please click here to view a larger version of this figure.

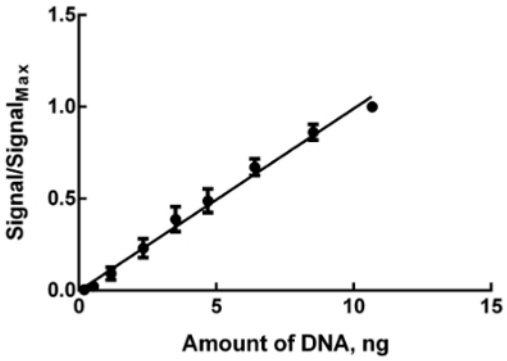

Figure 3. Calibration curve of DNA at 1500 bp using NBT-SG I: 12.5% polyacrylamide gels loaded with different concentrations of DNA ladder. Gels were run at 100 V for 2 h in 1x TAE. Please click here to view a larger version of this figure.

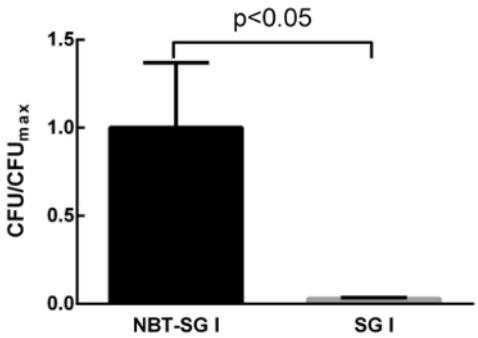

Figure 4. Comparison of transformation efficiency between DNA visualized with SG I and a transilluminator and NBT-SG I: The pDJ100 plasmid was loaded in a 1% agarose gel and run at 100 V for 1 h in 1x TAE. Then the gel was cut in two halves. One half was stained with SG I and other half of the gel with NBT-SG I. The plasmid band was then extracted from the gel using a gel extraction kit. Finally, the extracted plasmid was normalized and used to transform *E. coli* competent cells. The transformations were carried out n = 3. Please click here to view a larger version of this figure.

Discussion

A sensitive and novel DNA staining method based on the reduction of NBT and the use of SG I was presented in this article. The critical step in this protocol is the incubation time of the gel in the NBT-SG I solution and the concentration of NBT. The staining time for agarose gels is longer than for acrylamide gels because of the greater thickness of agarose gels. This method does not work well in the presence of SDS as NBT precipitates in contact with it. If the gel obtains a purple background, we recommend that another gel be prepared and to reduce the light exposure time. If an agarose gel is stained and there is not enough sensitivity, it is recommended to increase the light exposure time. If there is a lack of sensitivity and an opalescent precipitate is present in the gel, this could be the result of SDS contamination. In that case, prepare a new gel and rinse it before adding the staining reagents.

The precipitation occurs by blue light illumination, whether supplied by the light of a blue LED or the blue light present naturally in sunlight. Due to this, a limitation of the technique is the light source. If there no light source, a blue light source is needed, and if the light source is not homogeneous, it is not possible to do a comparison, and for the same reason, it is not possible to do quantification. However, the results obtained will be somewhat dependent on the light source used. If the light source is not consistent or uniform (*e.g.* due to changes in the intensity of sunlight during the day or non-homogeneous blue light source), it will be more difficult to do direct comparisons or quantification of different samples at different times.

This method does not need special instrumentation to visualize the DNA, making it ideal for field use. For quantitative results, one can use a high-resolution camera or an office scanner as was shown in this work.

We hope to expand this technique in the future by possibly combining it with the staining of other biomolecules through the use of two different staining techniques (*e.g.* NBT-SG I with Coomassie) in the same gel.

Disclosures

The authors have nothing to disclose.

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References

1. LePecq, J.B., Paoletti, C. A fluorescent complex between ethidium bromide and nucleic acids: physical-chemical characterization. *J. Mol. Biol.* **27** (1), 87-106 (1967).
2. Singer, V.L, Lawlor, T.E., Yue, S. Comparison of SYBR Green I nucleic acid gel stain mutagenicity and ethidium bromide mutagenicity in the Salmonella/mammalian microsome reverse mutation assay (Ames test). *Mutat Res.* **439** (1), 37-47 (1999).
3. Xin, X., Yue, S., Wells, K.S., Singer, V.L. SYBR Green-I: a new fluorescent dye optimized for detection picogram amounts of DNA in gels. *Biophys. J.* **66,** A150 (1994).
4. Flores, N., Valle, F., Bolivar, F., Merino, E. Recovery of DNA from agarose gels stained with methylene blue. *Biotechniques.* **13** (2), 203-205 (1992).

5. Yang, Y.I., Jung, D.W., Bai, D.G., Yoo, G.S., Choi, J.K. Counterion-dye staining method for DNA in agarose gels using crystal violet and methyl orange. *Electrophoresis.* **22** (5), 855-859 (2001).
6. Yang, Y.I*., et al.* Detection of DNA using a visible dye, Nile blue, in electrophoresed gels. *Anal. Biochem.* **280** (2), 322-324 (2000).
7. Santillán-Torres, J.L. A novel stain for DNA in agarose gels, *Trends Genet.* **9** (2), 40 (1993).
8. Adkins, S., Burmeister, M. Visualization of DNA in agarose gels as migrating colored bands: applications for preparative gels and educational demonstrations. *Anal. Biochem.* **240** (1), 17-23 (1996).
9. Cong, W.T*., et al.* A visible dye-based staining method for DNA in polyacrylamide gels by ethyl violet, *Anal. Biochem. 402.* (1), 99-101 (2010).
10. Altman, F.P. Tetrazolium salts and formazans. *Prog. Histochem. Cytochem.* **9** (3), 1-56 (1976).
11. Nineham, A.W. The chemistry of formazans and tetrazolium salts. *Chem. Rev.* **55** (2) 355-483 (1955).
12. Crandall, I.E., Zhao, B., Vlahakis, J.Z., Szarek, W.A. The interaction of imidazole-, imidazolium, and tetrazolium-containing compounds with DNA. *Bioorg. Med. Chem. Lett.* **23** (5), 1522-1528 (2013).
13. Paredes, A.J*., et al.* New visible and selective DNA staining method in gels with tetrazolium salts. *Anal. Biochem.* **517,** 31-35 (2017).
14. Green, M.R., Sambrook, J. *Molecular Cloning: a Laboratory Manual, fourth ed. Cold Spring Harbor Laboratory Press.* (NY), (2012).
15. Hansen, W., Garcia, P.D., Walter, P. *In vitro* protein translocation across the yeast endoplasmic reticulum: ATP-dependent post-translational translocation of the prepro-α-factor, *Cell.* **45** (3), 397-406 (1986).
16. Inoue, H., Nojima, H., Okayama, H. High efficiency transformation of Escherichia coli with plasmids. *Gene.* **96** (1), 23-28 (1990).
17. Madigan, M., Martinko, J., Parker, J. *Brock biology of microorganisms, 10th ed.*, *Prentice Hall/Pearson Education.*, Upper Saddle River (NJ) (2003).

