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Corresponding Author:	Todd Gruber UNITED STATES
Corresponding Author's Institution:	
Corresponding Author E-Mail:	todd.gruber@cnu.edu
Order of Authors:	Casey S. O'Neil
	Jacie L. Beach
	Todd D. Gruber
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TITLE:

DNA Electrophoresis Using Thiazole Orange Instead of Ethidium Bromide or Alternative Dyes

AUTHORS & AFFILIATIONS:

Casey S. O'Neil[1], Jacie L. Beach[1], Todd D. Gruber[1]

[1]Department of Molecular Biology and Chemistry, Christopher Newport University, Newport News, VA

Corresponding Author:

Todd D. Gruber

Email: todd.gruber@cnu.edu

Email Addresses of Co-authors:

Casey S. O'Neil (csoneil@terpmail.umd.edu)

Jacie L. Beach (jacie.beach.15@cnu.edu)

KEYWORDS:

gel electrophoresis, ethidium bromide, thiazole orange, agarose gel, DNA separation, DNA analysis

SUMMARY:

Here, we present a protocol to use thiazole orange for the detection of DNA in gel electrophoresis experiments. The use of thiazole orange allows elimination of ethidium bromide, and fluorescence detection can be achieved with either UV or blue light.

ABSTRACT:

DNA gel electrophoresis using agarose is a common tool in molecular biology laboratories, allowing separation of DNA fragments by size. After separation, DNA is visualized by staining. This article demonstrates how to use thiazole orange to stain DNA. Thiazole orange compares favorably to common staining methods, in that it is sensitive, inexpensive, excitable with UV or blue light (to prevent sample damage), and safer than ethidium bromide. Labs already equipped to run DNA electrophoresis experiments using ethidium bromide can generally switch dyes with no additional changes to existing protocols, using UV light for detection. Blue-light detection to avoid sample damage can additionally be achieved with a blue-light source and emission filter. Labs already equipped for blue-light detection can simply switch dyes with no additional changes to existing protocols.

INTRODUCTION:

The purpose of this method is to identify DNA in agarose gels using thiazole orange (TO) for fluorescence detection. Due to its low cost and favorable safety profile, thiazole orange may see particular benefit in undergraduate teaching labs and research labs performing molecular biology, especially ligations and cloning.

Ethidium bromide remains the most common dye for detection of DNA in agarose gels. This is

primarily because it can be obtained very inexpensively and only requires excitation with UV light for detection. Both ethidium bromide and thiazole orange are inexpensive, with low detection limits (1-2 ng/lane)[1]. There are two main drawbacks to ethidium bromide, however, that thiazole orange improves upon.

First, ethidium bromide is a mutagen[2] with special handling, shipping, and disposal requirements, whereas thiazole orange is less mutagenic (3–4x less mutagenic in Ames test)[3,4] and can be generally disposed of with common chemical waste.

Second, ethidium bromide requires UV light for detection. Thiazole orange can similarly use UV light if desired, but can also be detected with blue light. UV light, while commonly used, has a few salient disadvantages. First, it is damaging to human skin and eyes. While UV light can be used safely by trained professionals, accidental skin or eye damage (functionally similar to sunburns) from laboratory UV light are not uncommon particularly with inexperienced scientists. Second, UV light is extremely damaging to DNA samples[5], which reduces the success of downstream experiments (such as ligation and transformation)[1,6,7]. TO allows detection with blue light ($\lambda_{ex,max}$ = 510 nm (488 nm and 470 nm also show strong excitation)), which does not cause skin damage or DNA damage (although any intense light may still be harmful to eyes), greatly decreasing the risks to both the scientist and the sample.

TO is not the only fluorescent dye alternative to ethidium bromide; its advantage is cost. TO was discovered in the 1980s as a reticulocyte stain[8], and has found utility in a number of DNA-based fluorescence experiments[9-13]. It is currently sold by multiple suppliers. TO is the parent compound of additional, more expensive, blue-light–detectable commercial dyes, and behaves similarly during electrophoresis, using UV or blue light for detection[1]. Furthermore, while other dyes are more sensitive to very low DNA concentrations than either EtBr or TO, for generic electrophoresis experiments, such dyes are prohibitively expensive in many contexts.

PROTOCOL:

1. Preparing the gel

NOTE: For general gel electrophoresis protocols, see also P.Y. Lee, *et al*.[14].

1.1 Mix agarose (~1% w/v, percentage can be varied for particular size separations) in buffer (approximately 70 mL for a mini-gel (8 × 7 cm)). Buffers are commonly TAE (tris-acetate-EDTA, 40 mM Tris, 20 mM acetate, 1 mM EDTA, pH approximately 8.6) or TBE (tris-borate-EDTA, 90 mM Tris, 90 mM borate, 2 mM EDTA, pH approximately 8.3)).

1.2 Add thiazole orange to a final concentration of 1.3 μg/mL.

1.2.1 Dissolve thiazole orange in DMSO to make a 10,000x stock solution (13 mg/mL). While not particularly light sensitive, store TO in the dark when not in use. This solution is stable at room temperature for ~months; long-term storage may be achieved by freezing aliquots

(DMSO will freeze in a standard refrigerator). Be sure to entirely melt and resuspend a frozen solution before use.

NOTE: The gel can also be stained with TO after electrophoresis (see step 2.6). While TO has an improved safety profile over ethidium bromide, standard molecular biology laboratory safety precautions should be maintained.

1.3 Microwave the mixture of agarose, buffer, and thiazole orange to dissolve agarose (approximately 60 s). This step is commonly referred to as "melting". Swirl (5 s) to aid dissolution if needed.

NOTE: TO can also be added after microwaving if preferred.

1.4 Allow the agarose solution to cool briefly before pouring into gel casting apparatus containing an appropriate comb.

1.5 Allow the agarose solution to solidify into a gel.

2. Loading and running the gel

2.1 Place the gel in the electrophoresis apparatus if not already present.

2.2 Add running buffer (TAE or TBE as above) to cover the surface of the gel.

2.3 Load DNA samples (commonly 10 μL) using a loading dye. Include a DNA sizing ladder for reference.

2.4 Attach the cover and electrodes (the gel should be run toward the red anode (positive)).

2.5 Apply voltage (typically ~100V for a mini-gel, although size of gel may require a modified voltage to prevent damaging the gel) until loading dye has traveled an appropriate distance (approximately 4-7 cm for a mini-gel, although distance may vary depending on precise application).

NOTE: Like ethidium bromide and many other DNA-binding dyes, thiazole orange is positively charged. Consequently, these dyes will migrate in the opposite direction of electrophoresing DNA. For samples which are run far down the gel for enhanced separation, eventually the dye will separate from the smaller DNA fragments, resulting in weak staining. In these instances, the gel should be stained as in step 2.6. This situation is not unique to TO, any positively charged dye that reversibly interacts with DNA will exhibit this behavior (including ethidium bromide, TO, and others).

2.6 If TO was not added prior to gel casting (step 1.2), stain by immersing the gel in the buffer containing thiazole orange.

2.6.1 Prepare enough buffer (TAE or TBE) containing 1.3 μg/mL thiazole orange to completely cover the gel and soak the gel with gentle agitation until the bands are fully detected (roughly 20 min).

3. Visualization of thiazole orange agarose gel (UV transilluminator)

3.1 Remove the gel from the electrophoresis apparatus and place on a UV transilluminator.

CAUTION: UV light is damaging to skin and eyes. Be sure to wear appropriate eye (goggles) and face (face shield) protection. Hands should have gloves and long sleeves should be worn.

3.2 If desired, cut out desired DNA bands from the gel (for further digestion or ligation, for example). Expose the gel to UV light for as short a length of time as possible during excision. UV light (regardless of DNA dye) damages DNA.

3.3 Extract DNA from the gel slice using a readily available kit or protocol[15].

4. Visualization of thiazole orange agarose gel (blue-light transilluminator or flashlight)

4.1 Remove the gel from the electrophoresis apparatus and place on a blue-light transilluminator (~470 nm maximum emission wavelength). Alternatively, a blue LED (~470 nm) flashlight can be directed at the gel (either from above or below).

NOTE: While sensitivity is lower using a blue-light transilluminator with ethidium bromide, DNA stained with ethidium bromide can be detected using this blue-light protocol.

4.2 Use an amber emission filter (~560 nm longpass, either goggles or square) to filter blue light, enabling visualization of fluorescence from DNA:thiazole orange complexes.

NOTE: Without the amber emission filter, it is very difficult to detect DNA bands due to intensity of blue-light excitation source.

CAUTION: Although blue light lacks the ability to acutely damage tissues (contrasting UV), prolonged exposure to intense blue light could damage eyes and amber emission goggles or filter should be used.

4.3 If desired, cut out desired DNA bands from the gel for further applications.

NOTE: Since blue light does not damage DNA, it is not necessary to rapidly cut out bands (such as when using UV excitation in step 3.2).

4.4 Extract DNA from the gel slice using a readily available kit or protocol[15].

5. Image capture

5.1 Select appropriate excitation and emission settings in gel-imaging apparatus. The excitation and emission of thiazole orange ($\lambda_{ex,max}$ = 510 nm (488 nm and 470 nm also show strong excitation, in addition to strong excitation at UV wavelengths); λ_{em} = 527 nm) are nearly identical to common blue-light–detectable commercial dyes, so instruments may have preset filter settings that can be used.

NOTE: Some filter settings for blue-light–detectable commercial dyes actually use damaging UV light for excitation, so use caution if imaging prior to cutting out bands. Use a blue-light excitation source if possible when DNA bands will be excised after imaging.

5.2 In the absence of an imaging system with appropriate filters, place an amber filter between the camera and the gel/blue-light excitation source.

REPRESENTATIVE RESULTS:

Thiazole orange enables detection of DNA, without using ethidium bromide and without using DNA-damaging UV light. Ethidium bromide is well-known to be mutagenic, so eliminating it from the lab may be advantageous. UV light damages DNA and lowers transformation efficiency significantly, whereas blue light does not damage DNA. Detection limits are similar between ethidium bromide, thiazole orange, and a common, blue-light–detectable commercial DNA dye (**Figure 1,** see **Table of Materials**), with the detection limit for all three dyes being ~1-2 ng/lane in a mini-gel[1].

For common applications such as cutting out a restriction enzyme digested band, thiazole orange is particularly well-suited. Detection of DNA with blue-light excitation is robust and straightforward, and the scientist does not have to rush to excise DNA as they would if detecting with UV light. A plasmid was cut with restriction enzymes to isolate an insert **(Figure 2**, one gel is imaged three different ways). The insert is easily detectable with TO with blue light in addition to UV, allowing downstream applications to occur without fear of damage to the DNA from UV exposure.

FIGURE AND TABLE LEGENDS:

Figure 1. Detection of DNA using thiazole orange, a common blue-light–detectable commercial DNA dye, and ethidium bromide using blue or UV light. Gel slice images represent the two-fold dilution of a 120-ng band of DNA across the gel (band is a 3.0 kb band from 2-log ladder). This figure has been modified from O'Neil, et al.[1], reproduced with permission. See O'Neil, et al.[1] for complete details of the experiment.

Figure 2. Multiple images of the same thiazole orange-stained agarose gel of a restriction digest. (**A**) Excitation with UV transilluminator. (**B**) Excitation with blue-light transilluminator. (**C**) Excitation with blue-light flashlight (the tip of which is slightly visible, out of focus, in image at bottom). Lane 1: 2-log ladder, 1 μg of total DNA (major bands of 3.0 kb, 1.0 kb, and 0.5 kb are labeled). Lane 2: 0.5 μg of pEF-GFP plasmid DNA (5.1 kb) digested with HindIII (expected size

5.1 kb). Lane 3: 0.5 μg of pEF-GFP digested with HindIII and EcoRI (expected sizes: 3.7 kb, 1.3 kb). Gel was run with 1.3 μg/mL thiazole orange in the gel. All images taken using standard emission filter (590/110 nm), exposure optimized for intense bands.

DISCUSSION:

Ethidium bromide has long been a standard tool in the molecular biology lab, despite known toxicity. It also suffers from requiring UV light, which damages the DNA as it is being detected. Thiazole orange offers an inexpensive alternative to ethidium bromide, as well as useful but expensive commercial dyes.

The benefits of thiazole orange are thus two-fold. First, thiazole orange can simply be used as a replacement to ethidium bromide. Gels can be prepared identically to EtBr, with TO substituted as the stain (step 1.2). Detection limits are similar (~1-2 ng/lane)[1]. No additional equipment is required to switch dyes because thiazole orange can be detected with UV light (step 3) just like EtBr. Exposure to UV light rapidly damages DNA, however, and may cause failure of downstream experiments such as ligation and transformation[1]. UV light is also damaging to skin and eyes, requiring careful safety precautions.

The second benefit of TO is that it offers the possibility of shifting away from UV excitation. Detection with blue light (replacing step 3 with step 4) eliminates damage to DNA and limits risk to the scientist. Blue-light excitation and detection of TO can be achieved with a blue-light transilluminator, and also with an inexpensive blue LED flashlight (both ~470 nm maximum emission wavelength, both requiring an amber emission filter). Appropriate application of excitation wavelengths (step 4.1) and emission filters (step 4.2) is essential to the success of the experiment (see also step 5.1). Light sources and emission filters are readily available, however, and with minimal investment, labs can gain the benefits of blue-light excitation and avoid UV light damage.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure 1

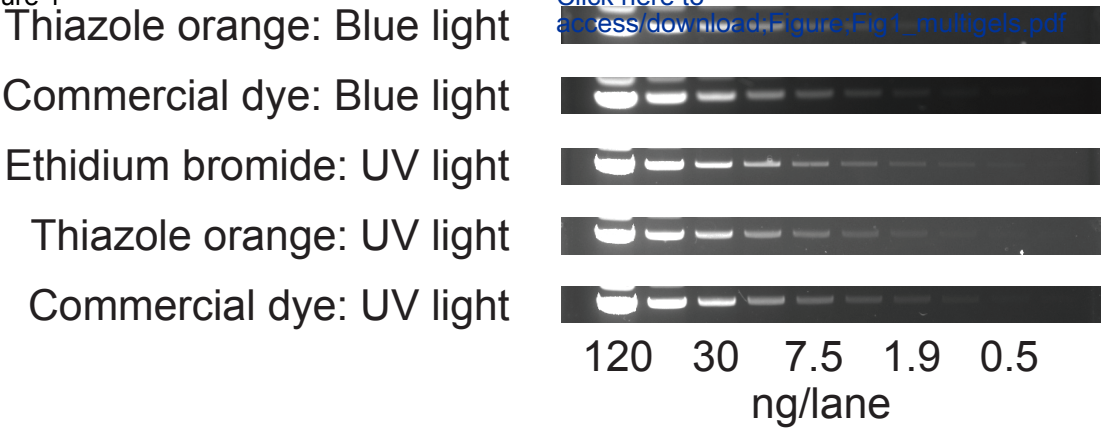
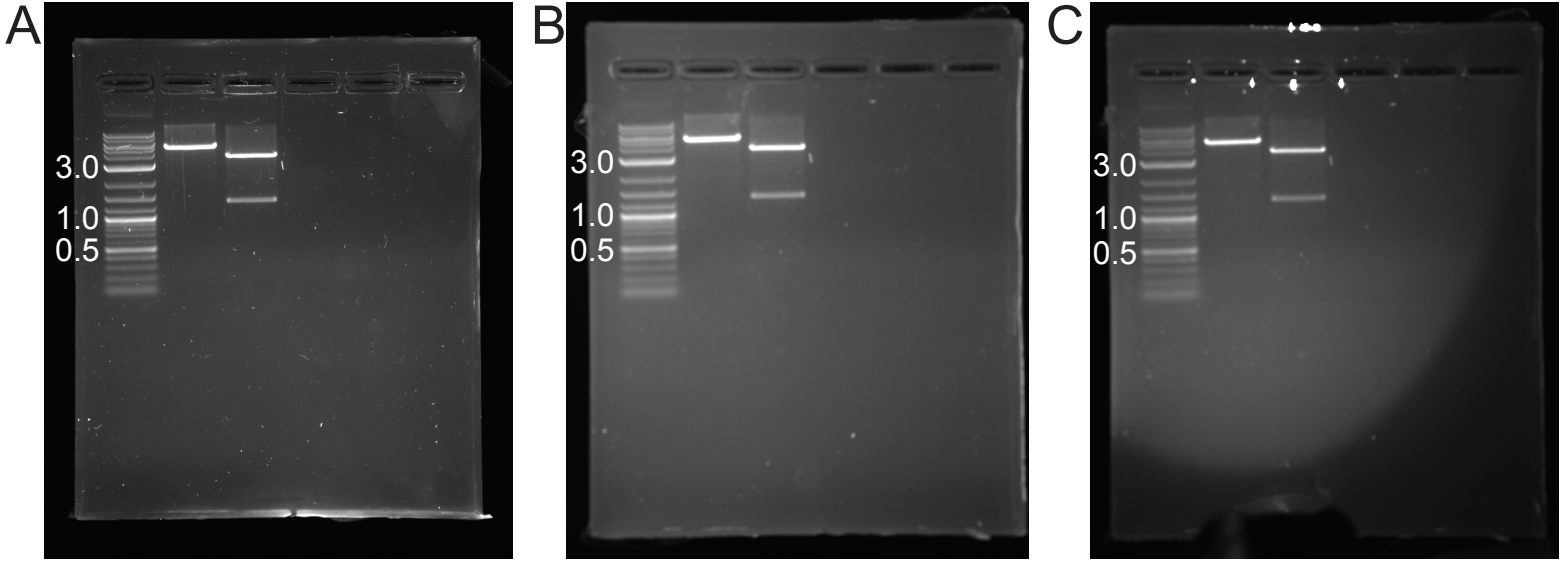


Figure 2

Click here to access/download;Figure;Fig2_gelsv2.pdf



Name of Material/ Equipment	Company	Catalog Number	Comments/Description
2-log DNA ladder	New England Biolabs	N0469S	
Agarose (Genetic Analysis Grade)	Fisher	BP1356-100	
Blue-light flashlight	WAYLLSHINE (Amazon)	WAYLLSHINE Scalable Blue LED	
ChemiDoc MP	Biorad	1708280	
DMSO	Sigma-Aldrich	D8418	
ethidium bromide	Fisher	BP1302-10	For comparison, not necess
Gel apparatus (Owl Easy Cast)	Thermo Scientific	B1A	
Qiagen Qiaquick Gel extraction kit	Qiagen	28704	
Safe Imager Viewing Glasses	Invitrogen	S37103	Necessary for using blue lig
SafeImager 2.0 (Blue light transilluminator)	Invitrogen	G6600	Blue light flashlight may be
SYBR Safe	Invitrogen	S33102	For comparison, not necess
TAE (Tris-Acetate-EDTA)	Corning	46-010-CM	
Thiazole orange	Sigma-Aldrich	390062	

*Glasses are also included with Invitrogen G66(

sary for protocol

ht flashlight.*

used as alternative

sary for protocol

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CORRESPONDING AUTHOR:

Name:	Todd D. Gruber
Department:	Molecular Biology and Chemistry
Institution:	Christopher Newport University
Article Title:	DNA electrophoresis using thiazole orange instead of ethidium bromide
Signature:	Todd D. Gruber
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3. Please define all abbreviations before use, e.g., TAE, TBE, etc.

Done.

4. Please use h, min, s for time units.

Done.

5. Step 1.2.2: This step should be written as a note.

Done.

6. 1.3: Dissolve agarose in what? How much agarose is used? How long is it microwaved and swirled?

Done. Details have been added and the text has been modified to clarify that the step refers to the agarose mixture made previously in step 1.1.

7. 2.4: How much sample is added?

Done.

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To whom it may concern,

I recently published an article in Electrophoresis:

O'Neil, C. S., Beach, J. L., Gruber, T. D., ELECTROPHORESIS 2018, 39, 1474–1477.

I would like to submit the method used in the paper to the Journal of Visualized Experimentation (JoVE). I would like to use parts of Figure 2 in the new submission. What do I need to do to obtain permission to do this?

Thank you very much,

Todd Gruber

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Todd D. Gruber, Ph.D.

Assistant Professor

Department of Molecular Biology and Chemistry

Christopher Newport University

Office: (757) 594-7123

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