Focal Points

Application Note FP-170

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Safe Gel Imaging with Blue Light Excitation Using GelGreen™ Dye with the GelDoc-It® Imaging System and Visi-Blue™ Converter Plate

Introduction

By using state-of-the-art dyes and gel imaging systems, researchers can achieve repeatable, highly detailed documentation while reducing exposure to toxic materials and UV radiation during preparative gel handling.

Gel imaging and nucleic acid binding dyes are widely used in today's life science laboratories to visualize DNA fragments in agarose gels. Ethidium bromide (EtBr) has been the predominant dye used for nucleic acid gel staining for decades because of its low initial price and generally sufficient sensitivity (1, 2). However, the safety hazards and costs associated with decontamination and waste disposal can ultimately make the dye comparatively expensive and unsafe to use. For this reason, safer alternative gel stains were developed by scientists at Biotium, Inc. GelGreen™ dye is a next-generation of fluorescent nucleic acid gel stain. Three attributes make GelGreen dye superior to EtBr and other EtBr alternatives: low toxicity, high sensitivity and exceptional stability. GelGreen offers the added advantage of imaging using visible blue light instead of potentially harmful UV illumination.

Figure 1: UVP's GelDoc-It Imaging System

GelGreen is a nucleic acid binding dye that can be precast in agarose gels or used to stain gels after electrophoresis**.** Once nucleic acid samples are separated by electrophoresis and stained, the GelDoc-It Imaging System (UVP, LLC, **Figure 1**) images the fluorescent bands using the UVP FirstLight® UV Transilluminator with 302nm UV excitation, the Visi-Blue blue light converter plate, and a green fluorescence emission filter (**Figure 2**). The Visi-Blue converter plate converts 302nm UV light to 460-470nm blue light. The use of the Visi-Blue converter plate both protects researchers from exposure to harmful UV irradiation during band excision from preparative gels and prevents UV damage to DNA which can dramatically reduce subsequent cloning efficiency (3). Furthermore, the GelCam 310 2.0 megapixel camera used in this application is ideal for high resolution imaging of stained gels.

Materials and Methods

Agarose gel preparation
Molten agarose was prepared by mixing agarose with 1X TBE at a final concentration of 1% and microwaving until it dissolved completely. Molten agarose was cast in an OWL gel electrophoresis system (Thermo Fisher Scientific Inc.) and allowed to solidify and cool for approximately one hour.

DNA samples
DNA ladders were obtained from the following suppliers and loaded in the amounts (total mass) as indicated: 1) 1 kB Ladder (Biotium, 200ng), 2) 1 kB Plus DNA Ladder (Invitrogen 200ng), 3) GeneRuler™ 1kB Ladder (Fermentas, 200 ng), 100bp Ladder (New England BioLabs, 200 ng), HyperLadder™ IV (Bioline, 30 ng), 1 kb DNA Ladder (Axygen, 175 ng).

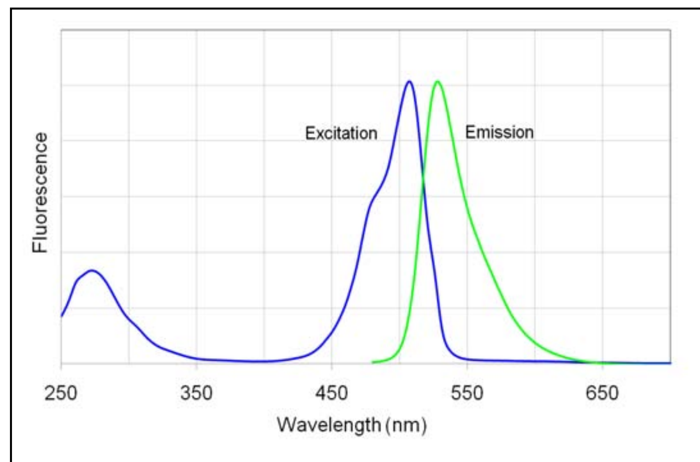

Figure 2: Excitation and emission spectra of GelGreen dye bound to dsDNA.

DNA ladders were mixed with 6X DNA loading buffer (7.5% Ficoll, 15% glycerol, 0.1% Patent Blue VF, 0.05% Bromophenol Blue; 2 uL 6X loading buffer + 10 uL DNA ladder) before loading onto gels.

Gel electrophoresis
Electrophoresis was performed in 1X TBE at 100V until tracking dyes had migrated half the length of the gel.

Gel staining
After electrophoresis, gels were stained in 3X GelGreen (Biotium) in water for 30 minutes before imaging.

Gel imaging
Gels were imaged using the GelDoc-It Imaging System equipped with the FirstLight UV Transilluminator equipped with a Visi-Blue blue light converter plate (UVP, LLC). The FirstLight UV Transilluminator provides less than a 5% coefficient of variance (CV), creating highly uniform transillumination. Gels were placed on the Visi-Blue converter plate which was placed atop the transilluminator surface for imaging. Images were acquired at 0.25 to 2 second exposure times with the VisionWorks®LS image acquisition and analysis software (UVP, LLC).

Cell membrane permeability studies
Cell staining procedures investigating the membrane permeability of dyes were performed in HeLa cells cultured in DMEM supplemented with 10% BCS and antibiotics. Cells were incubated in 1X concentrations of SYBR Safe (Invitrogen) or GelGreen diluted from 10,000X stocks. Microscopic images of cells were captured using an Olympus America, Inc. mercury arc lamp microscope and Image-Pro® Express software (Media Cybernetics, Inc.).

Results

DNA fragments in GelGreen post-stained gels were imaged on the GelDoc-It Imaging System using the Visi-Blue converter plate and amber emission filter. Gels were documented and pseudocolored green using the VisionWorksLS acquisition and analysis software (**Figure 3**).

To evaluate the membrane permeability of GelGreen dye compared to SYBR Green and SYBR Safe, HeLa cells were stained with GelGreen, SYBR Green or SYBR Safe DNA gel stains at 1X concentration at 37°C. SYBR Green and SYBR Safe readily penetrated cells and stained DNA within five minutes, while no nuclear staining was evident with GelGreen after 30 minutes of staining (**Figure 4**).

Discussion

Traditionally, imaging of EtBr gels using tube-based UV transilluminators and film has been the means to detect and document nucleic acid bands in gels. However, newer technologies such as safer, brighter and simpler nucleic acid binding dyes and imaging systems that incorporate the GelCam 310 camera, highly uniform transilluminators such as the FirstLight, and analytical software outperform imaging with traditional EtBr and film.

While GelGreen is compatible with UV transilluminators, the dye was developed primarily to meet the needs of researchers who use systems employing visible blue light for excitation. Blue light illumination protects researchers from exposure to UV light from transilluminators which burns skin and eyes and can act as a carcinogenic (4). In addition, visible light illumination protects DNA from UV damage during DNA band excision from preparative gels which has been reported to reduce subsequent cloning efficiency by as much as 100-1000-fold (3). Furthermore, GelGreen is also compatible with downstream applications such as sequencing and cloning.

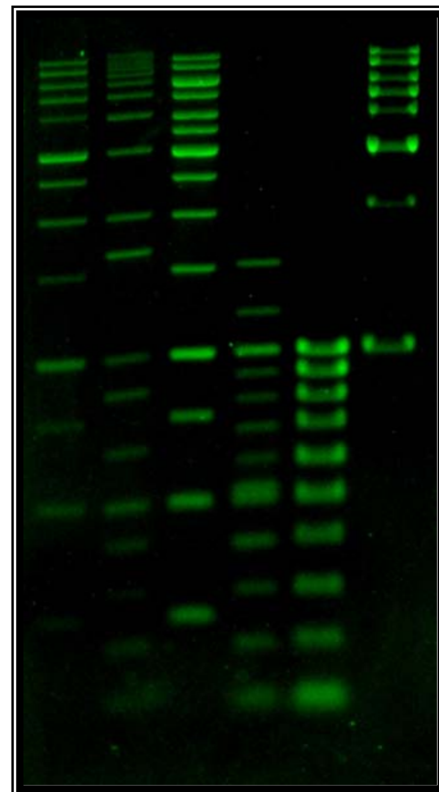

Figure 3: GelGreen post-stained gel imaged using the Visi-Blue converter plate. DNA ladders were separated on a 1% agarose TBE gel and stained in 3X GelGreen in water. DNA ladders from the following suppliers were loaded in the amounts (total mass) indicated from left to right: 1) 1 kB Ladder (Biotium, 200ng), 2) 1 kB Plus DNA Ladder (Invitrogen 200ng), 3) GeneRuler 1kB Ladder (Fermentas, 200 ng), 4) 100bp Ladder (New England BioLabs, 200 ng), 5) HyperLadder IV (Bioline, 30 ng), 6) 1 kb DNA Ladder (Axygen, 175 ng). Images were taken on the GelDoc-It Imaging System equipped with the GelCam 310 camera, FirstLight UV Transilluminator, Visi-Blue converter plate & amber emission filter. Images were captured and pseudocolored green using VisionWorksLS software.

GelGreen dye has been shown to be less toxic than both SYBR Green and ethidium bromide. The genotoxicity of DNA-binding dyes can be substantially reduced by preventing dye binding to genomic DNA in living cells. Therefore, Biotium scientists engineered the chemical structure of GelGreen such that the dye is incapable of crossing the plasma membrane of viable cells. In contrast, SYBR dyes, including SYBR Safe, penetrate living cells rapidly and stain mitochondria and nuclear DNA **(Figure 4)**, making it more likely for the dyes to be toxic and mutagenic. Indeed, SYBR Green has been reported to strongly potentiate DNA mutation by UV light and other genotoxic agents (5). Standard Ames tests conducted by independent laboratories have confirmed that GelGreen dye is nonmutagenic and noncytotoxic at concentrations well above working concentrations. Furthermore, environmental safety tests show that GelGreen dye is nonhazardous and nontoxic to aquatic life. GelGreen successfully passed the Aquatic Toxicity Test (CCR Title 22) based on the EPA/600/4-85/013 protocol (for more information, please refer to the Safety Report of GelRed and GelGreen, also available at www.biotium.com).

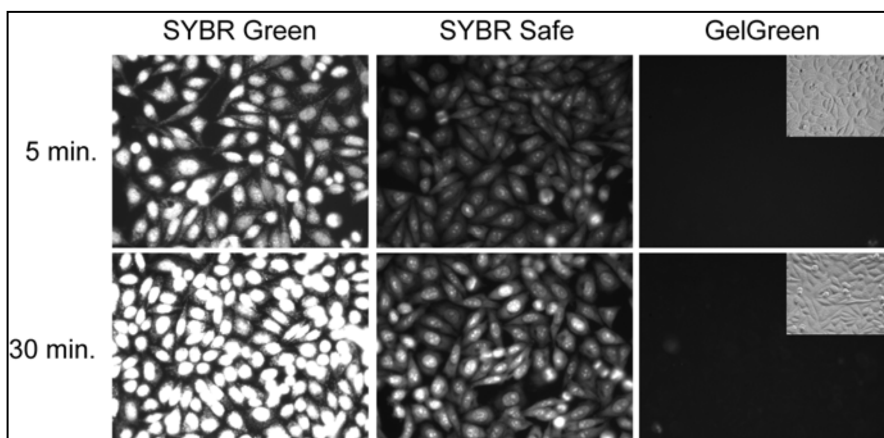

Figure 4: Comparison of membrane permeability of SYBR Green, SYBR Safe, GelGreen. HeLa cells were incubated for 30 minutes at 37ºC with 1X SYBR Green, 1X SYBR Safe, or 1X GelGreen dyes. SYBR Green and SYBR Safe entered cells rapidly as evidenced by the bright green fluorescent nuclear staining after five minutes incubation. However, GelGreen dyes were unable to cross cell membranes as shown by the absence of fluorescence staining even after 30 minutes incubation. Insets: phase contrast images of fluorescence field of view.

In addition, GelGreen dye offers superior sensitivity and chemical stability over other green nucleic acid binding dyes such as SYBR Safe or SYBR Green. GelGreen 10,000X concentrated dye stock is available formulated in water instead of DMSO, is chemically stable and photostable, and can be stored at room temperature. GelGreen can be used for post-staining as described herein, or mixed directly with molten agarose for preparation of pre-cast GelGreen gels for immediate imaging of DNA after electrophoresis (**Figure 5**; for more information, please refer to the GelGreen product protocol, also available at www.biotium.com).

The FirstLight UV Transilluminator offers a unique patented design emitting 302nm ultraviolet excitation, combining a specially designed, high density grid array lighting configuration with a phosphor coating to generate exceptionally uniform ultraviolet illumination. It produces less than a 5% coefficient of variance (CV) across the full imaging surface, essential for capturing high quality images for documentation and quantitative analysis. The FirstLight UV Transilluminator's design assures consistent sensitivity and dynamic range for achieving accurate and reproducible gel analysis no matter where the gel is placed on the surface. The Visi-Blue converter plate is simply placed on top of the FirstLight UV Transilluminator box to convert 302nm UV light to 460-470nm blue illumination for convenient and flexible imaging. In addition, the digital, high resolution, GelCam 310 CCD camera offered with the GelDoc-It Imaging System is a huge step above traditional film documentation in both quality and cost per image.

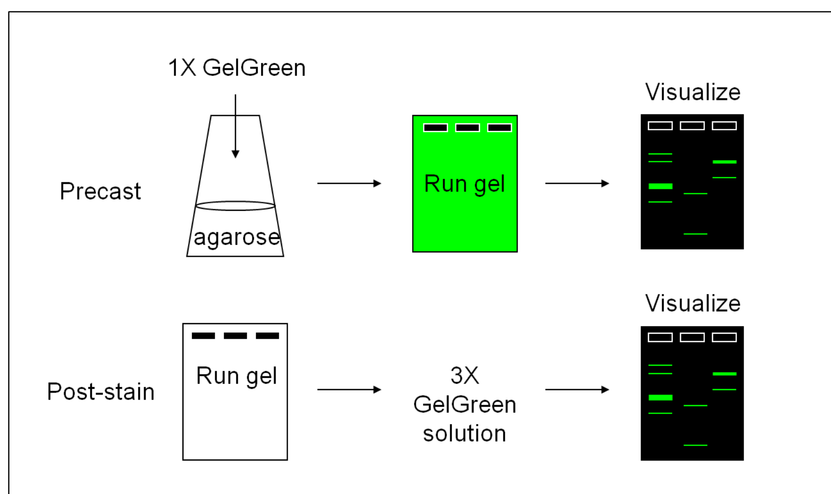

Figure 5: Two methods for using GelGreen Nucleic Acid Stain. Overview of precast and post-staining procedures.

Conclusion

Innovative technologies such as Biotium's nucleic acid binding dyes and UVP's advanced imaging systems allow for highly sensitive imaging documentation and analysis. Biotium's GelGreen Dye is safer for researchers and the environment compared to other DNA dyes, while Visi-Blue transillumination protects both researchers and their DNA samples from damaging UV rays. These systems, in conjunction with top-quality reagents and software, minimize effort while maximizing informative results and molecular biology workflow efficiency in today's life science laboratories.

References

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