# Focal Points

## Application Note FP-153

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# Next Generation Gel Imaging Using GelRed™ and GelGreen™ Dyes with the GelDoc-It® Imaging System

## Introduction

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

*GelDoc-It Imaging System*

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 hazard and costs associated with decontamination and waste disposal can ultimately make the dye expensive and unsafe to use. For this reason, safer alternative gel stains were developed by scientists at Biotium, Inc. GelRed™ and GelGreen™ dyes are a new generation of fluorescent nucleic acid gel stains designed to replace the highly toxic EtBr. Three attributes make GelRed and GelGreen dyes superior to EtBr and other EtBr alternatives: low toxicity, high sensitivity, and exceptional stability.

GelRed and GelGreen dyes are nucleic acid binding dyes that can be precast in agarose gels or used to stain gels after electrophoresis (**Figure 1**). Once nucleic acid samples are separated by electrophoresis and stained, the GelDoc-It® Imaging System (UVP, LLC) images the fluorescent bands using the UVP FirstLight® UV Illuminator to excite the fluorescence with 302 nm UV and visualize the sample with the appropriate green or red filter. The GelCam 310 2.0 megapixel camera used in this application is ideal for high resolution imaging for stained gels.

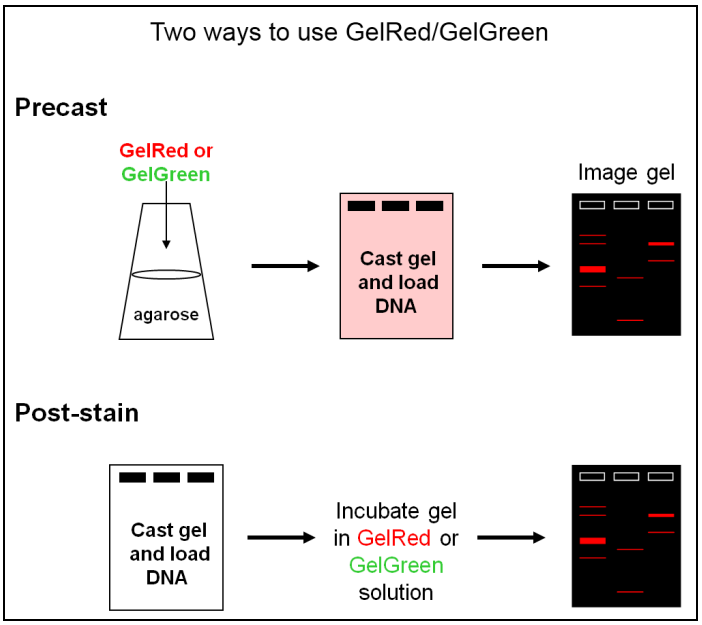


***Figure 1.*** *Two methods for using GelRed and GelGreen dyes. Overview of precast and post-staining procedures.*

## 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 about 1 hour.

***Preparation of precast GelRed/GelGreen gels***

For precast GelRed or GelGreen gels, 10,000X dye stock in water was added to molten agarose to a final concentration of 1X and mixed well before casting. No GelRed or GelGreen was added to gels used for post-staining with GelRed and GelGreen.

***DNA samples***

DNA ladders were obtained from the following suppliers: 1) 1kb ladder (Biotium), 2) 1kB Plus DNA Ladder (Invitrogen), 3) GeneRuler™ 1kB Ladder (Fermentas), 4) 100bp Ladder (New England BioLabs).

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 100 V until tracking dyes had migrated half the length of the gel. Pre-cast GelRed/GelGreen gels were imaged immediately following electrophoresis.

***Post-electrophoresis gel staining***
DNA samples were loaded on agarose gels containing no fluorescent nucleic acid dye. After electrophoresis, gels were stained in 3X GelRed or GelGreen in water for 30 minutes before imaging.

***Gel imaging***
Gels were imaged using a GelDoc-It Imaging System equipped with the 302 nm FirstLight UV Illuminator (UVP, LLC) for uniform illumination, GelCam 310, and EtBr and green emission filters. Images were typically acquired at 0.25 to 2 second exposure times with the VisionWorks®LS 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), GelRed 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.).

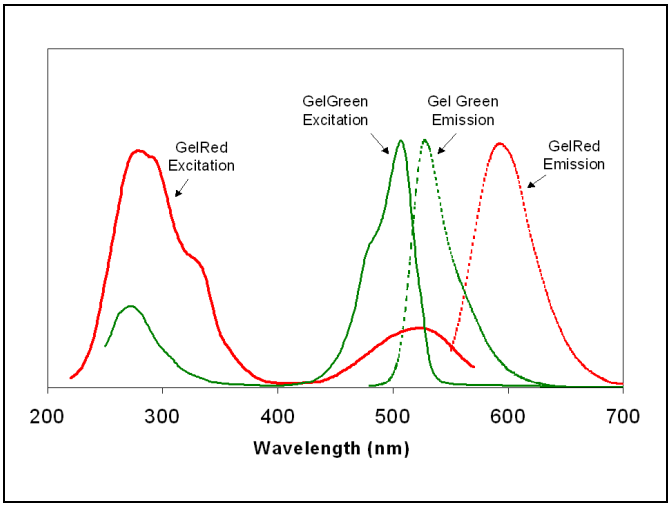


***Figure 2.*** *Excitation and emission spectra of GelRed and GelGreen dyes bound to dsDNA.*

## Results

Designed primarily for use with a 302 nm UV transilluminator, GelRed dye is spectrally similar to EtBr. GelGreen dye is also compatible with UV transilluminators but was developed to meet the needs of researchers who use a 488 nm laser-based gel scanner or systems that use visible blue light for excitation. Excitation and emission of GelRed and GelGreen dyes make it optically compatible with UV transilluminators and other documentation systems (**Figure 2**).

DNA fragments in GelRed and GelGreen post-stained gels were imaged on the GelDoc-It imaging system and documented using the VisionWorks LS image acquisition and analysis software (**Figure 3**). Images of GelRed gels were obtained using the EtBr emission filter and pseudocolored red while images of GelGreen gels were obtained using the green emission filter and pseudocolored green.

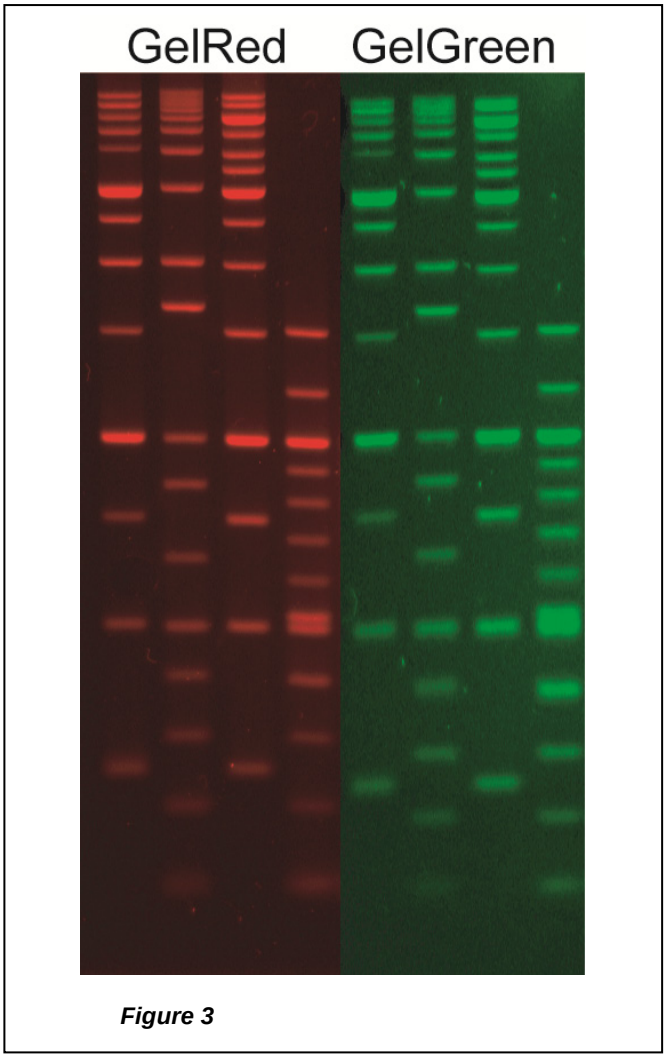


***Figure 3***

***Figure 3.*** *GelRed and GelGreen post-stained gels. DNA ladders were separated on a 1% agarose TBE gel and stained in 3X GelRed or 3X GelGreen in water. Samples in the lanes are as follows: 1) 1kb ladder (Biotium), 2) 1kB Plus DNA Ladder (Invitrogen), 3) GeneRuler 1kB Ladder (Fermentas), 4) 100bp Ladder (New England BioLabs). The total DNA mass loaded in each well was 200 ng. Images were taken on a GelDoc-It system equipped with the FirstLight UV transilluminator, GelCam 310 Camera, and EtBr emission filter (GelRed) or green emission filter (GelGreen) using the VisionWorks LS software and pseudocolored red or green.*

To compare the sensitivity of GelRed and EtBr, precast agarose gels were loaded with two-fold dilutions of 1 kB Plus DNA Ladder (Invitrogen). EtBr migrates through gels toward the anode during electrophoresis, resulting in poor staining of low molecular weight bands and high background in the region of high molecular weight bands **(Figure 4**).

GelRed in precast gels does not migrate as readily as EtBr during electrophoresis, resulting in more uniform staining of high and low molecular weight bands. A band containing approximately 2 ng of DNA can be clearly detected using GelRed (**Figure 4**), demonstrating that DNA fragments in the nanogram range are readily detectable using GelRed dye and the GelDoc-It system. The fluorescence intensity of the dye combined with the excitation and imaging capacity of the GelDoc-It system allow for highly sensitive detection of nucleic acids.

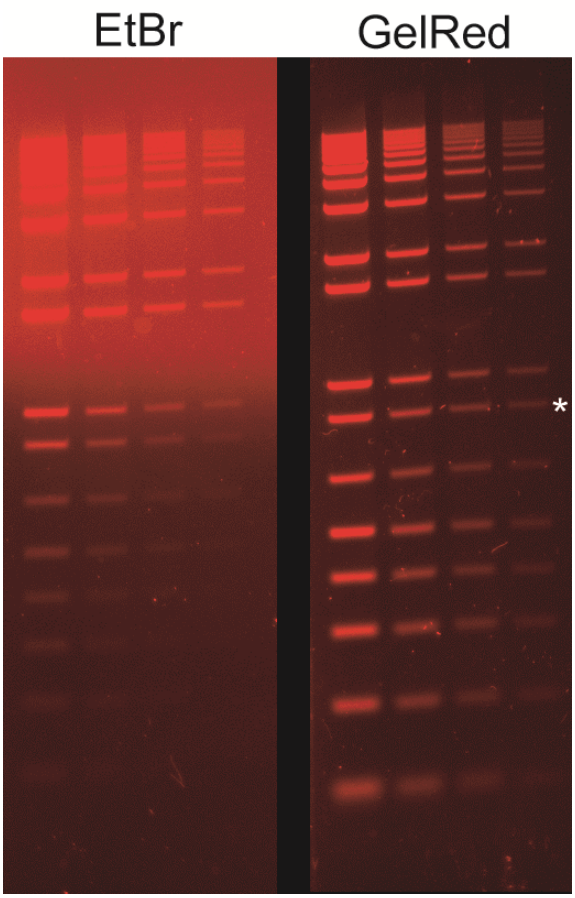


***Figure 4**. Comparison of EtBr and GelRed in precast gels. Two-fold serial dilutions of 1 kb Plus DNA Ladder (Invitrogen) were loaded in the amounts of 200 ng, 100 ng, 50 ng and 25 ng per well from left to right. Images were taken on a GelDoc-It system equipped with a FirstLight 302 nm transilluminator, EtBr and green emission filter. Gel images were captured using the GelCam 310 and VisionWorks LS software and pseudocolored red. The band in the far right lane marked by the asterisk (*) contains approximately 2 ng DNA.*

***Figure 4***

To demonstrate the safety of GelRed and GelGreen dyes, the membrane permeability of the dyes was assessed. HeLa cells were stained with SYBR® Safe DNA gel stain, GelRed and GelGreen dyes at 1X concentration from 10,000X stocks. SYBR Safe readily penetrated the cells and stained DNA while no nuclear staining was evident with GelRed and GelGreen (**Figure 5**).

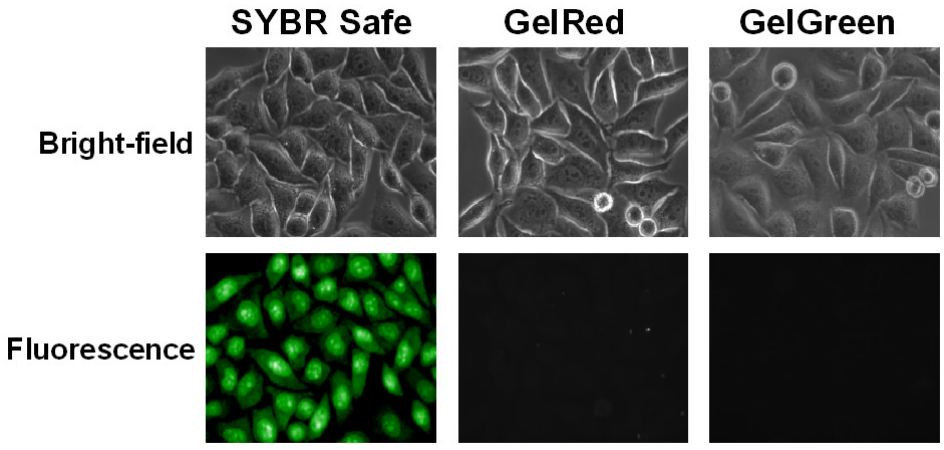


***Figure 5.** Comparison of membrane permeability of SYBR Safe, GelRed, and GelGreen. HeLa cells were incubated for 30 minutes at 37ºC with 1X SYBR Safe, GelRed, and GelGreen dyes. SYBR Safe entered cells rapidly as evident from the bright green nuclear staining. However, GelRed and GelGreen dyes were unable to cross cell membranes as shown by the absence of fluorescence staining.*

## 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 simple to use nucleic acid binding dyes and imaging systems that incorporate GelCam 310 camera, uniform illuminators, and analytical software outperform imaging with EtBr and film.

GelRed and GelGreen dyes have been shown to be less toxic and more sensitive than EtBr and SYBR Safe. The genotoxicity of DNA-binding dyes can be substantially reduced by preventing dye binding to genomic DNA in living cells. Thus, Biotium scientists engineered the chemical structures of GelRed and GelGreen such that the dyes are 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 5)**, making it more likely for the dyes to be toxic and mutagenic. Indeed, SYBR Green I has been reported to strongly potentiate DNA mutation by UV light and other genotoxic agents (3). Standard Ames tests conducted by independent laboratories have confirmed that GelRed and GelGreen dyes are nonmutagenic and noncytotoxic at concentrations well above their working concentrations. Furthermore, environmental safety tests showed that GelRed and GelGreen dyes are nonhazardous and nontoxic to aquatic life. GelRed and GelGreen successfully passed the Aquatic Toxicity Test (CCR Title 22) based on the EPA/600/4-85/013 protocol (please visit www.biotium.com to download a detailed safety report on GelRed and GelGreen).

GelRed and GelGreen offer several additional advantages over EtBr and other nucleic acid dyes. When used in precast gels, GelRed does not migrate through the gel as easily as EtBr; therefore, there is less disparity between high molecular weight and low molecular weight staining intensities (**Figure 4**), and it is not necessary to add the dyes to the running buffer for maximal sensitivity. Also, unlike EtBr, destaining after post-staining with GelRed is not necessary due to the low intrinsic fluorescence of GelRed dye when not bound to nucleic acids. GelGreen dye offers superior sensitivity and chemical stability over other green nucleic acid binding dyes such as SYBR Safe or SYBR Gold and allows for visible light excitation for those who wish to minimize UV exposure to themselves and their DNA samples (**Figure 2**). GelRed and GelGreen are highly stable at room temperature for long-term storage. Both dyes are also very photostable, permitting their use under normal room light without exercising special precaution. In addition to 10,000X GelRed and GelGreen in water, 3X GelRed in water (4L unit size) is available for post-staining applications **(Figure 1).**

The FirstLight Illuminator offers a unique patented design emitting 302 nm ultraviolet excitation and combines a specially designed, high density grid array ultraviolet lighting configuration with a phosphor coating to generate exceptionally uniform ultraviolet illumination. It produces less than 5% coefficient of variance (CV) across the full imaging surface, which is essential for capturing high quality images for documentation and quantitative analysis. The FirstLight Illuminator design assures consistent sensitivity and dynamic range for achieving accurate and reproducible gel analysis no matter where the gel is placed on the surface.

In addition, the digital, high resolution, GelCam 310 camera offered with the GelDoc-It Imaging System is a step above traditional film documentation.

## 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. These systems, in combination with top-quality reagents and software, minimize effort and maximize informative results in today's life science laboratories.

## References

1. Gallagher, S.R. and Wiley, E.A. Current Protocols: Essential Laboratory Techniques. 2008.
2. Armstrong, J and Schulz, J. 2008. Agarose Gel Electrophoresis. *Curr. Protoc. Essential Lab. Tech.* Unit 7.2.
3. Ohta T, Tokishita S, and Yamagata H. 2001. Ohta T, Tokishita S, and Yamagata H. 2001. Ethidium bromide and SYBR Green I enhance the genotoxicity of UV-irradiation and chemical mutagens in *E. coli*. Mutation Res. **492**, 91.



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