Focal Points

Application Note FP-153

MIDSCI 800.227.9997 • 636.227.9997 • Gel Doc Equipment

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

Introduction

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

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 to use. For this reason, alternative, safer 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 used either as precast or post-gel stains. The 10,000X stock solutions of GelRed and GelGreen dyes are routinely used to cast agarose gels or as poststains after electrophoresis. More recently, prestaining kits further reduce dye use and increase usable results and repeatability. The prestaining kits offer a 6X sample loading buffer conveniently containing GelRed and GelGreen dye and a superior proprietary running buffer.

The 6X loading buffers also contain colorimetric tracking dyes for easy visualization of the migration front, and the running buffer formulation has been especially formulated for running gels at high voltages without overheating. Samples are simply mixed with the optimally formulated GelRed and GelGreen loading buffer, loaded into the gel and run with the proprietary running buffer.

Once the 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 is ideal for high resolution imaging for stained gels and is used here.

GelDoc-It Imaging System

Materials and Methods

The GelRed and GelGreen prestaining kits were used throughout. Agarose (0.7 – 3%) was microwaved in GelRed and GelGreen 1X running buffer until it dissolved completely, and cast in an OWL gel (Thermo Fisher Scientific Inc.) electrophoresis system. 6X GelRed and GelGreen loading buffer was premixed with DNA ladders (1:6 dilution; 2uL 6X loading buffer + 10uL sample) before loading onto the gels. DNA ladders were commercially purchased and are as follows: (1) Fermentas GeneRuler™ 1kB ladder, (2) Invitrogen 1kB Plus DNA Ladder™, (3) New England BioLabs Lambda DNA-HindIII digest, (4) Bioline HyperLadder™ I, (5) Bioline HyperLadder™ IV, (6) Axygen M-DNA-LR, (7) Axygen M-DNA-BR, (8) Fermentas GeneRuler™ Ultra Low Range ladder, (9) Invitrogen 1kB ladder, and (10) Promega Lambda-HindIII digest. Gels were typically run in GelRed or GelGreen 1X running buffer at 160 V for 1 to 2 hours.

Gel images were taken using a GelDoc-It 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 taken at 0.25 to 2 second exposures with the VisionWorks® LS (UVP, LLC) software.

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, 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.). SYBR Safe was commercially purchased from Invitrogen Corp.

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. The excitation and emission of GelRed and GelGreen dyes make it optically compatible with UV transilluminators and other documentation systems (Fig. 1).

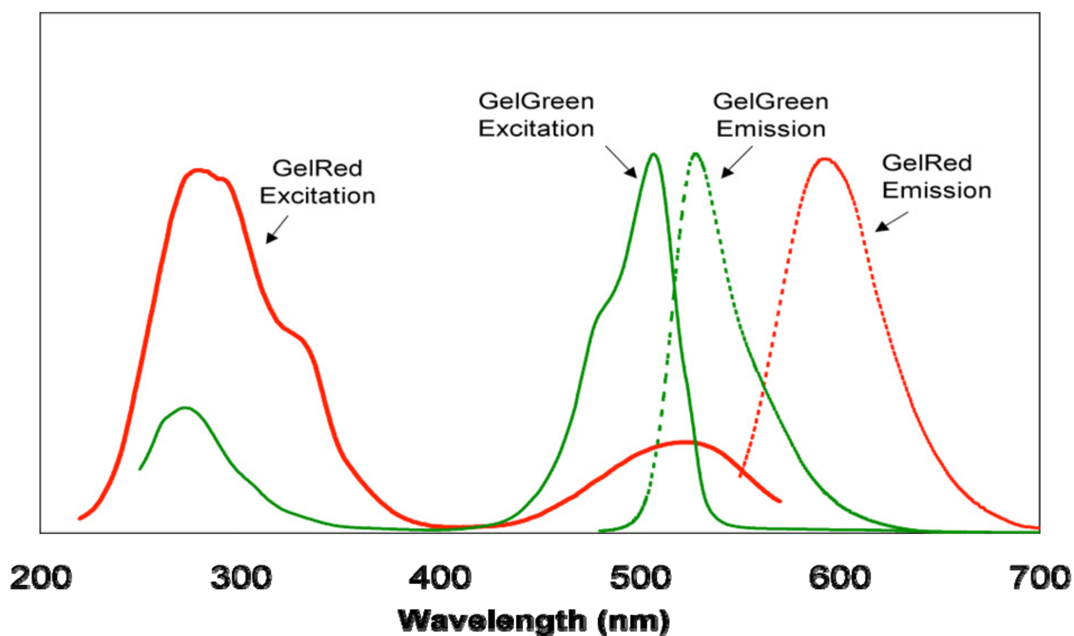

Figure 1. Excitation and emission spectra of GelRed™ and GelGreen™ dyes bound to dsDNA.

Visualization of DNA fragments stained with GelRed and GelGreen prestaining loading buffers was achieved on the GelDoc-It imaging system (**Figure 2**). DNA ladders from several different vendors were premixed with GelRed and GelGreen loading buffer, electrophoresed in a 1% agarose gel, and documented using the VisionWorksLS image acquisition and analysis software. Images of GelRed gels were taken with the EtBr emission filter and pseudocolored red while images of GelGreen gels were taken with the green emission filter and pseudocolored green using the software.

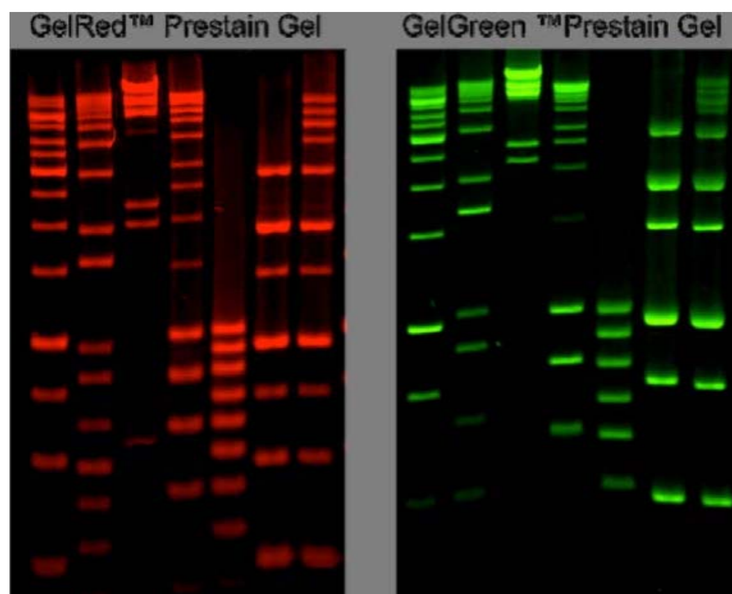

Figure 2. GelRed™ and GelGreen™ prestain gels. DNA ladders were mixed with GelRed or GelGreen loading dye and electrophoresed in 1X GelRed/GelGreen running buffer. Samples in the lanes are as follows: (1) Fermentas GeneRuler ™1kB ladder, (2) Invitrogen 1kB Plus DNA Ladder™, (3) New England BioLabs Lambda DNA-HindIII digest, (4) Bioline HyperLadder™ I, (5) Bioline HyperLadder™ IV, (6) Axygen M-DNA-LR, and (7) Axygen M-DNA-BR. Total DNA in each well ranged from 100 to 500 ng. Images were taken on a GelDoc-It system equipped with the FirstLight UV transilluminator, GelCam 310, and EtBr and green emission filters. Images were pseudocolored red or green using the VisionWorks®LS software.

A 1 ng band of DNA can be clearly seen after performing a titration of the GeneRuler Ultra Low Range DNA Ladder (**Figure 3**). Thus, Figure 3 demonstrates that GelRed dye and the GelDoc-It system are able to detect DNA fragments easily in the nanogram range. The fluorescence intensity of the dyes combined with the excitation and imaging capacity of the GelDoc-It system allow for highly sensitive detection of nucleic acids.

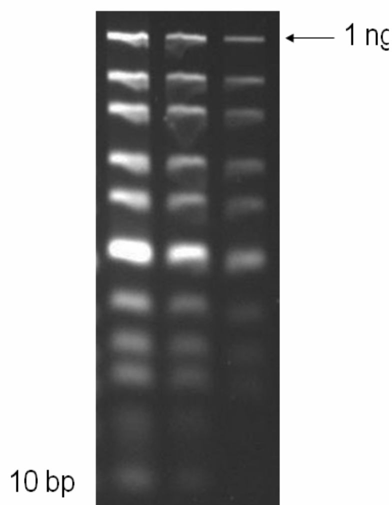

Figure 3. GelRed™ loading buffer was premixed with Fermentas GeneRuler™ Ultra Low Range DNA Ladder at 62.5, 31.3, and 15.6 ng per lane from left to right, and run on a 3% agarose gel. Images were taken on the GelDoc-It system. The lowest band is a 10 bp DNA fragment. The uppermost band in the right lane is approximately 1 ng. Images were taken on a GelDoc-It system equipped with a FirstLight 302 nm transilluminator, EtBr and green emission filter. Gel images were captured with a GelCam 310 and VisionWorksLS software.

To compare the prestaining intensities of GelRed and EtBr or GelGreen and SYBR Safe dyes, agarose gels were loaded with several commercially available DNA markers. GelRed or EtBr **(Figure 4A)** and SYBR Safe or GelGreen dyes **(Figure 4B)** were mixed with DNA ladders at the same final concentration, loaded and run on a gel.

Although EtBr and SYBR Safe are not typically used for prestaining protocols, GelRed and GelGreen dyes are far more sensitive than their counterparts in this application. Figure 4 demonstrates that prestaining with EtBr or SYBR Safe is difficult and may require very high concentrations of these dyes for maximal sensitivity.

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 the same 1X concentration from 10,000X stocks. SYBR Safe readily entered the cells and stained DNA while no nuclear staining was evident with GelRed and GelGreen dye.

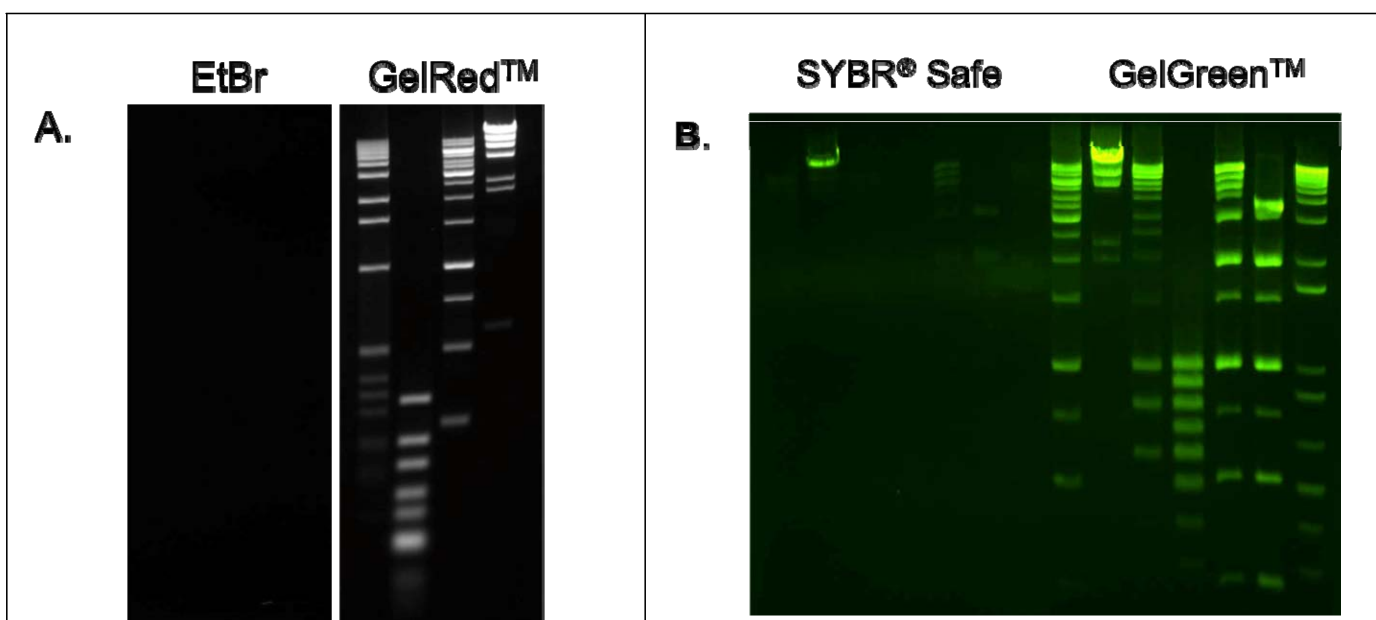

Figure 4. DNA ladders were mixed with EtBr or GelRed™ dye (A), or SYBR® Safe or GelGreen™ dye (B) before loading onto the gel and electrophoresed as described in the methods. Samples in the lanes for (A) are as follows from left to right: (1) Invitrogen 1kB ladder, (2) Fermentas GeneRuler™ Ultra Low Range ladder, (3) Fermentas GeneRuler™ 1kB ladder, and (4) Promega Lambda-HindIII digest. Samples in the lanes for (B) are as follows: (1) Fermentas GeneRuler™ 1kB ladder, (2) New England BioLabs Lambda DNA-HindIII digest, (3) Bioline HyperLadder™ I, (4) Bioline HyperLadder™ IV, (5) Axygen M-DNA-BR, (6) Axygen M-DNA-LR, and (7) Invitrogen 1kB Plus DNA Ladder™. Images were taken on the GelDoc-It imaging system.

Discussion

Up until now, EtBr, in conjunction with tube based UV transilluminators and film, have been the means to detect and document bands in gels. However, newer technologies, such as safer, brighter, and simple to use nucleic acid binding dyes and imaging systems that incorporate GelCam 310s, uniform illuminators, and analytical software outperform imaging with EtBr and film.

Other EtBr-alternative dyes have reduced mutagenicity, but they achieve this by sacrificing features of dyes. For example, SYBR Safe sacrifices sensitivity. SYBR Green and SYBR Gold (Invitrogen Corp.) dyes are much less stable than EtBr. SYBR dyes also enter cells rapidly to stain mitochondria and nuclear DNA **(Figure 5)**, making it more likely for the dyes to be toxic at high enough concentrations. Indeed, SYBR Green I is known to strongly potentiate mutation caused by UV light or another mutagen (3).

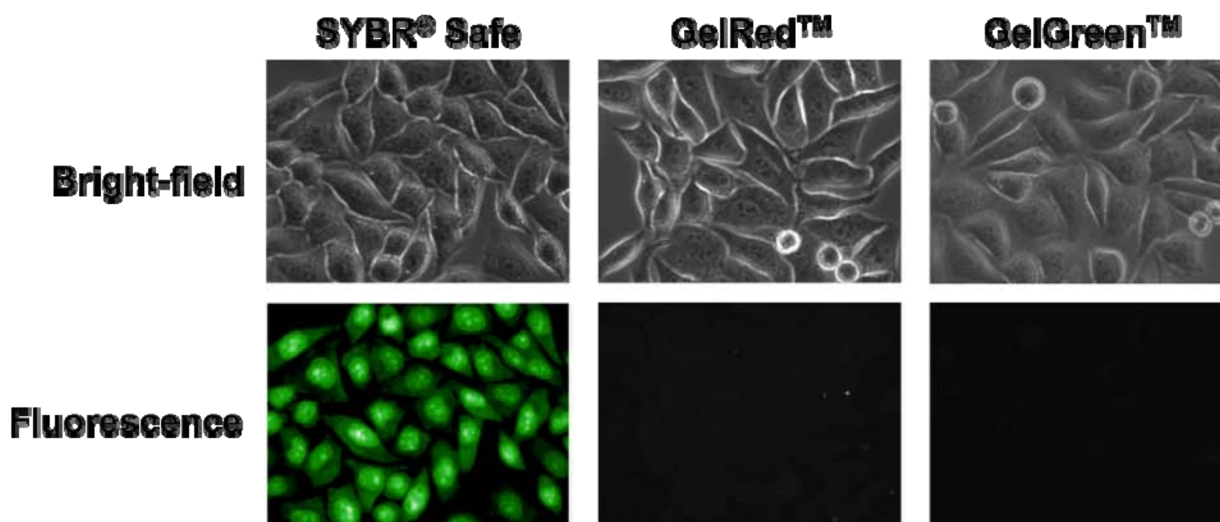

Figure 5. HeLa cells were incubated at 37ºC with 1X of SYBR® Safe, GelRed™, and GelGreen™ dyes, respectively. Images were taken following incubation for 30 minutes. SYBR Safe entered into cells rapidly as evident from the bright green nuclear staining. However, GelRed and GelGreen dyes were unable to cross cell membranes as shown by the lack of any fluorescence staining.

GelRed and GelGreen dyes have been shown to be less toxic and more sensitive than EtBr and SYBR Safe. A DNA-binding dye can be made nonmutagenic or substantially so by denying its chance to be in contact with genomic DNA in living cells. Thus, Biotium scientists engineered the chemical structures of GelRed and GelGreen dye such that the dyes are incapable of crossing cell membranes of live cells (Figure 5). A standard Ames test 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.

In addition to the prestaining kits, 10,000X concentrated stocks of GelRed and GelGreen dyes in water are available for precast and poststaining applications **(Figure 6).** They can be added to the molten agarose before casting or used to incubate gels after electrophoresis.

GelRed and GelGreen dyes offer several additional advantages over EtBr and other nucleic acid binding dye alternatives. GelRed dye in precast methods 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, and it is not necessary to add the dyes to the running buffer for maximal sensitivity. Also, unlike post-staining with EtBr where destaining is necessary, the low intrinsic fluorescence of GelRed dye does not require any destaining after post-staining gels. GelRed dye is only highly fluorescent once bound to nucleic acids. GelGreen dye offers superior sensitivity and stability over other nucleic acid binding dyes such as SYBR Safe or SYBR Gold and allows for visible light excitation for those concerned with limiting UV excitation (Figure 1). The GelRed and GelGreen dyes are perfectly 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.

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.

The digital, high resolution, GelCam 310 offered with the GelDoc-It imaging system is a step above traditional film documentation.

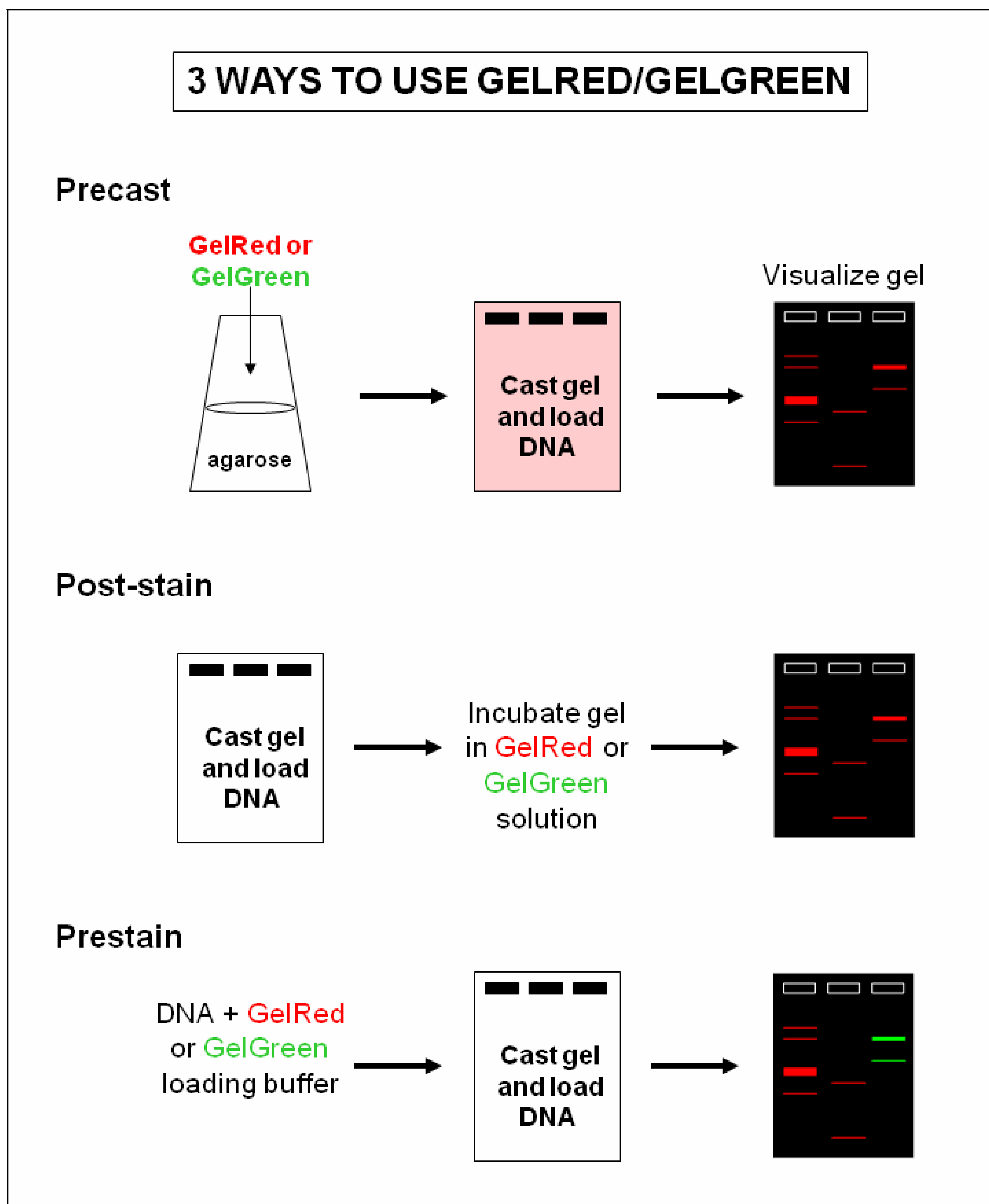

Figure 6. Three methods for using GelRed™ and GelGreen™ dyes. Overview of precast, poststaining, and prestaining procedures.

Conclusion

Innovative technologies such as Biotium's nucleic acid binding dyes and UVP's 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

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