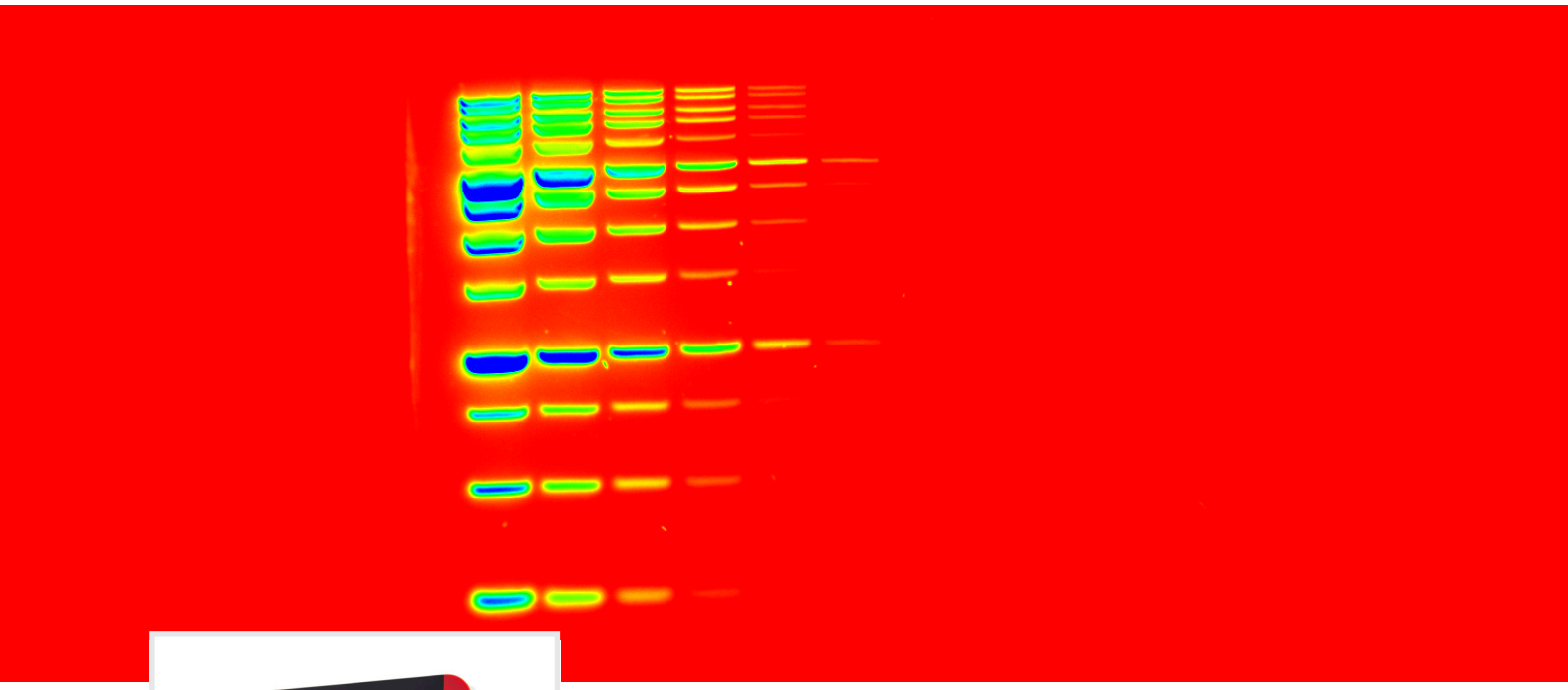

Task

Safer DNA gel imaging

Solution

User-centered built-in safety features in all of Analytik Jena's UVP Studio systems combined with Biotium's GelRed and GelGreen safe DNA dye technology

Safety is a Part of Science: Considerations for Safer DNA Gel Imaging

Introduction

Gel electrophoresis is a common procedure in life science laboratories to identify and separate nucleic acid fragments for a variety of downstream applications. As such, routine imaging of gels has become a fast and easy approach to visualizing results. The tools required for these techniques are regularly improved upon to enable simpler protocols, higher sensitivity, and safer handling/disposal of reagents. Recent advances in dye technology enable greater sensitivity and safety. Historically, DNA binding dyes used in gel imaging have safety warnings stemming from their reported toxicity and mutagenicity. Ethidium bromide (EtBr) is a ubiquitous dye used in laboratories that is cost-effective and sensitive. Ethidium bromide works by intercalating double stranded DNA and fluorescing upon exposure to ultraviolet (UV) light. Unfortunately, the inherent capacity of EtBr to bind DNA confers its toxic and mutagenic properties.[1,3] Because EtBr is cell permeable, mutagenesis can occur by frameshift or base substitution mutations upon cell uptake. Safety hazards and disposal concerns with EtBr have prompted the development of new alternatives.

In this application note, Biotium GelRed® and GelGreen® nucleic acid binding dyes are tested against traditional and more hazardous alternatives through DNA gel electrophoresis, and are then evaluated on the UVP GelStudio to enable high-resolution image capture. The UVP GelStudio provides the researcher with an enclosed darkroom with limited UV exposure. In addition, the researcher can monitor adjustments in real-time through an on-board touch display. In addition, researchers can use a VisiBlue® UV-to-Blue light

converter plate or a Blue LED to eliminate the risks of UV altogether. Lastly, the UVP GelStudio has an easy access filter tray to enable emission filter swapping, giving researchers maximum flexibility in accommodating safety without compromising on quality. Below, we demonstrate that GelRed® and GelGreen® are high-performance dyes for gel imaging that can be used with UV or blue excitation light. With safety as a central tenant in the design of our instruments and reagents, Analytik Jena and Biotium are helping researchers make a shift towards safer alternatives for themselves and the communities they serve.

Materials and Methods

Agarose Gel Preparation

Agarose gels were prepared by mixing J.T. Baker low electroendosomosis agarose (Radnor, PA) and 1X TBE from a 50X TAE Thermo Scientific stock solution (Waltham, MA) to a concentration of 0.8% and microwaving until completely dissolved. Biotium gels were prepared in the same manner, except Biotium GelRed® and GelGreen® Agarose LE (Fremont, CA) were used. Molten agarose was poured into a Wealtec mini-gel electrophoresis system (Sparks, NV). Wells were formed using an 8-well 1.5mm thick comb.

DNA Binding Dyes

Since Biotium GelRed® and GelGreen® Agarose LE already contain dye, Thermo Fisher's UltraPure™ Ethidium Bromide, SYBR™ Safe, and SYBR™ Green (Waltham, MA) were added directly to the molten agar, per the manufacturer's instructions, and mixed on a stir plate to homogeneity. By mixing each stain into the agarose, any staining bias associated with post-staining practices is eliminated.

DNA Samples

Biotium's Ready-to-Use 1kb DNA ladder was added to each gel in a 1:2 dilution series from 400ng-3ng. Samples were diluted in Biotium's 6X DNA Loading Dye, pre-diluted to 2X.

Gel Electrophoresis

All gels were run at 5V/cm for approximately 90 minutes or until the loading buffer dye reached the bottom of the gel.

Gel Imaging

Gels were imaged on the UVP GelStudio equipped with RGB light emitting diodes and 302nm UV light sources. Excitation light sources and emission filters were selected based on the peak excitation and emission of the dyes shown in Table 1. Exposure time was set to 1.090 seconds. No post-processing was done on any of the images, aside from pseudocoloring using VisionWorks software. Colors were used to help distinguish between the dye emission wavelengths, and heatmapping was applied to highlight signal-to-noise distribution within the gel.

Table 1: Excitation and emission data for Ethidium Bromide, GelRed®, GelGreen®, SYBR™ Safe, and SYBR™ Green.

Dye	Extraction Source (nm)	Peak Excitation (nm)	Peak Emission (nm)	Filter Peak Wavelength (nm)	Filter Bandpass (nm)
Ethidium Bromide	302 (UV)	210/285	605	605	50
GelRed®	302 (UV)	279	593	605	50
GelGreen®	456 (Blue LED)	507	528	535	20
SYBR™ Safe	302 (UV)	280/502	530	535	20
SYBR™ Green	456 (Blue LED)	497	520	535	20

Results and discussion

DNA was visible using all dyes after gel electrophoresis to varying degrees (Figure 1). The most striking results was the high background noise present in the EtBr (Figure 1A, 1F) and SYBR™ Safe gels (Figure 1C, 1H), and the paucity of noise in SYBR™ Green (1B, 1G), Biotium GelRed® (1D, 1I), and GelGreen® (1E, 1J) gels. GelRed® and GelGreen® dyes do not migrate as easily through the agarose gel as EtBr, resulting in less disparity in staining intensity between high molecular weight and low molecular weight fragments. This phenomenon is most obvious in intensity heatmaps (Figure 1, compare 1F to 1I, 1J). GelRed® and GelGreen® also inherently have less noise when compared to EtBr, due to low intrinsic fluorescence when not bound to nucleic acids, thus eliminating post-electrophoresis destaining, which is required for EtBr and SYBR™ Safe (Figure 1I ,1J). Considerations for the safety of nucleic acid binding dyes is an emerging trend, as researchers become more aware of the environmental hazards dyes pose for aquatic and marine life, as well as the toxic and mutagenetic risks posed to researchers. Modern dyes have been enhanced for not only better sensitivity, but also to overcome environmental and personnel risks. Using the Ames test, GelRed® and GelGreen® were confirmed to be non-toxic and non-mutagenic at concentrations well above their working concentrations[4]. GelRed® and GelGreen® dyes are incapable of crossing the plasma membranes of viable cells[2], eliminating their potential to be toxic or mutagenic to cells unlike SYBR™ Green[2], which had comparable sensitivity (Figure 1B, 1G). These results highlight GelRed® and GelGreen® as the true safest and environmentally sensible choice for researchers.

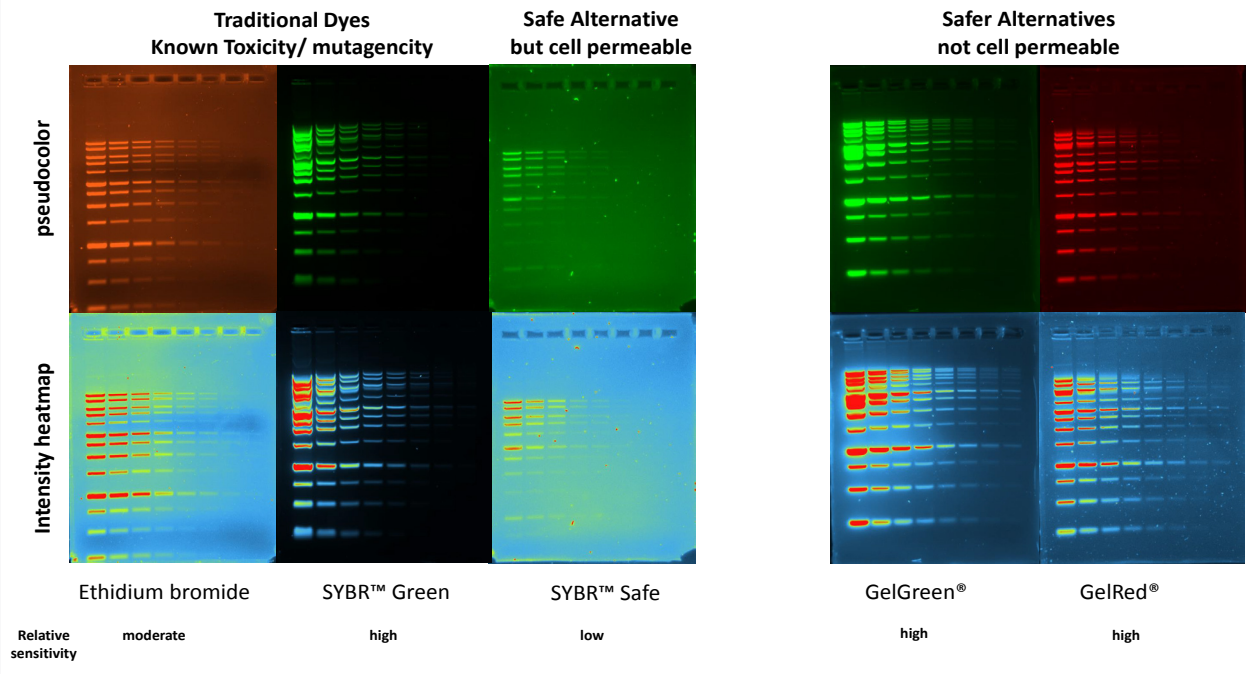

Figure 1: A comparison of gels imaged using the UVP GelStudio imaging system and pseudocolored using VisionWorks software.

Concluding Remarks

Equipped with a 5 megapixel CMOS camera, multiple excitation sources, and filter options that cover the entire visible spectrum, the UVP GelStudio imaging system provides high-quality images with robust flexibility for the researcher. Furthermore, as researchers ourselves, safety for personnel is at the heart of our designs with several built-in safeguards for the end user. When combined with sensibly designed dyes, Analytik Jena and Biotium form a symbiotic mutualism to provide safe, high-quality instruments and reagents for the research community.

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