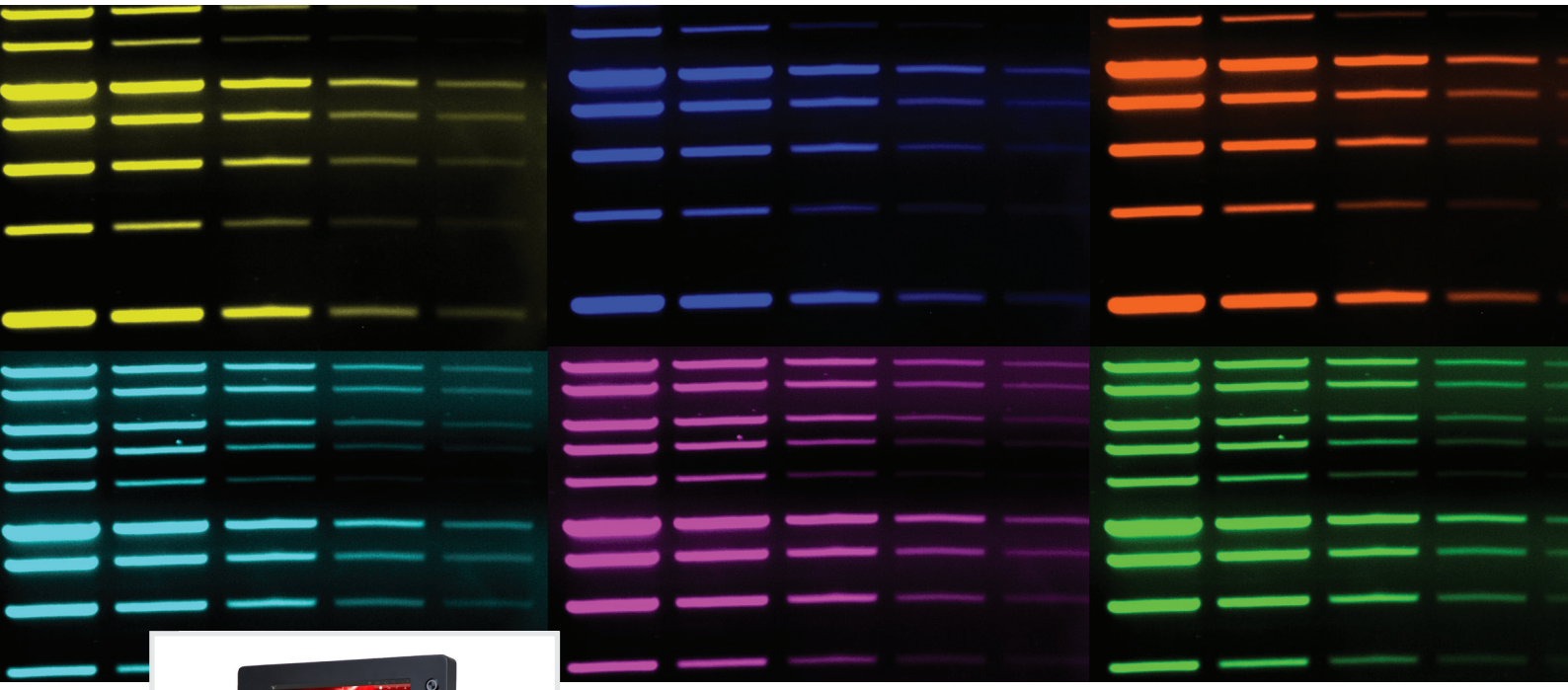

Task

Acquire gel images easily and without any need for training

Solution

Simple user interface and one touch automation enables researchers and students to acquire DNA and protein gel sample images without training

Simplified Gel Documentation

Introduction

Gel electrophoresis serves as a confirmation that the right nucleic acid product was generated during an experiment. Applications include nucleic acid fragment analysis after restriction enzyme treatment, checking successful PCR, or testing for genes associated with a disease of interest. Agarose gel itself is a permeable matrix that allows charged nucleic acid fragments to migrate within the gel towards an opposite charge. The gel is placed in an electrophoresis chamber filled with buffer that can conduct an electric current, which causes the nucleic acid to move. Smaller fragments travel through the gel quicker than larger fragments that cannot move as far in the same timeframe. This movement ultimately separates the fragments by size, creating a series of bands that can then be analyzed.
Gel imaging is required to visualize the bands in a gel by detecting fluorescent signals of nucleic acid binding dyes mixed into the gel or used to stain the gel post-electrophoresis. As a widely used technique, gel imaging systems and a variety of binding dyes are commonplace in life sciences laboratories. One such imaging system, the UVP GelSolo, is built for high-resolution imaging optimized for maximum light sensitivity and quick capture speeds in low-light or long exposure applications. With an 8-48mm manual lens and up to three UV wavelength options, the UVP GelSolo provides the flexibility to detect a broad range of fluorescent wavelengths.
Ethidium bromide (EtBr) has been the predominant dye used for nucleic acid gel staining because of its low initial cost and adequate sensitivity[1,2]. However, due to the safety hazards and additional costs associated with decontamination and waste disposal of EtBr, more and more laboratories have moved to safer alternatives. GelRed and GelGreen are two such alternatives developed by Biotium, Inc to address safety concerns with EtBr while also enhancing dye sensitivity.

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In this application note, a demonstration of how gel imaging is accomplished in a research setting is presented using an experimental simulation. A silicone gel containing plastic fluorescent rods will mimic a stained agarose gel. The gel will be imaged using the UVP GelSolo system, showing how the nucleic acid binding dyes GelRed and GelGreen appear in a captured image. This application note can be used by non-research institutions as a teaching tool to supplement STEM curriculum. Further training can be accomplished with those who may have access to imaging instruments using the same method to create and image a silicone gel.

Materials and Methods

DNA Binding Dye Simulation

GelRed is considered a nontoxic alternative dye that is more environmentally friendly than EtBr. Yet, the spectrum of GelRed is similar to that of EtBr, so the same excitation sources and emission filter can be used for both dyes (Figure 1.). In this experiment, GelRed dye bound DNA fragments are represented by red plastic fluorescent rods in the silicone gel.

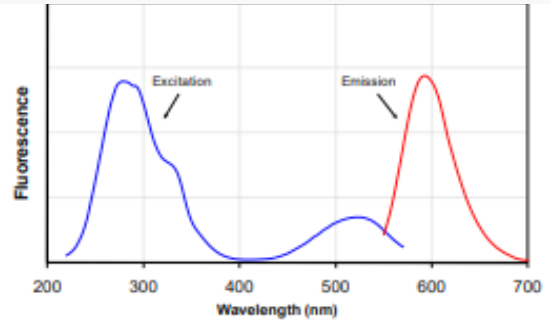

Figure 1. a) GelRed Excitation and Emission spectrum.
Source: (GelRed: Biotium)

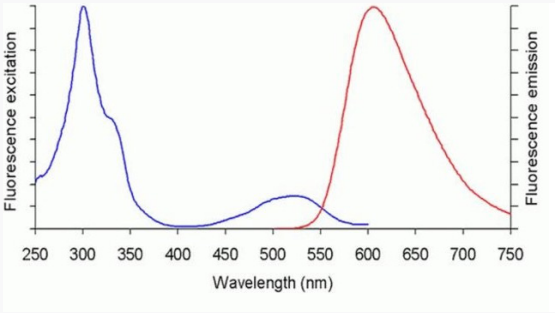

Figure 1. b) EtBr Excitation and Emission spectrum
Source: (EtBr: ThermoFisher)

GelGreen is another nontoxic dye option within a different wavelength range. The GelGreen spectrum is displayed in Figure 2. For this experiment, GelGreen bound DNA fragments will be represented by plastic yellow-green fluorescent rods. Both GelRed and GelGreen can be excited using 302nm UV. The emission filter range used for GelRed is 575-640nm and for GelGreen is 513-557nm.

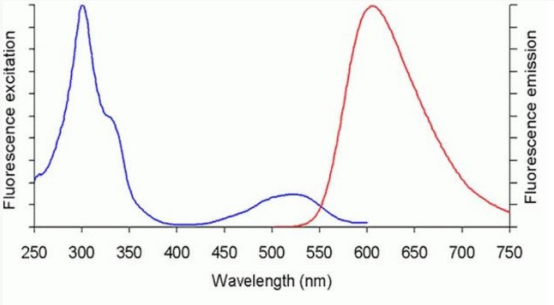

Figure 2. GelGreen spectrum (Source: Biotium)

Silicone DNA Gel

The silicone DNA Gel is made from semi-transparent silicone rubber. The red and yellow-green plastic fluorescent rods were lined up in columns on the surface of the solid silicone rubber. A second layer of liquid silicone was poured over the rods and dried. The resulting silicone gel is approximately 5mm thick, which is the recommended thickness for standard agarose gels used in DNA electrophoresis. If the agarose gel is thicker than 5mm, there will be lower resolution of the bands and higher background from staining.

Silicone Gel Imaging

The silicone gels were imaged in the UVP GelSolo imaging system equipped with a 5.0-megapixel 315 camera and 8-48mm f/1.2 manual zoom lens. To detect GelRed and GelGreen wavelengths, 575-640nm and 513-557nm emission filters were placed in the filter slot located on top of the system. Table 1 details the image capture data for each color dye.

	Exposure Time	Filter
Red Fluorescent (GelRed)	110ms	575nm-640nm
Green Fluorescent (GelGreen)	60ms	513nm-557nm

Image Acquisition Step-by-Step Procedure

1. Turn on the UV transilluminator and switch the UV option to 302nm.
2. Double click to start VisionWorks software installed on the embedded touch computer.
3. Choose the DNA Gel Electrophoresis application and start a preview by clicking the Live View button.
4. Manually adjust the aperture, zoom and focus on the manual lens until the image looks sharp and clear.
5. Click Capture to capture the image.

Results

The UVP GelSolo definitively detected both the GelRed and GelGreen wavelengths, represented here by red and green fluorescent rods strategically placed in the silicone gels (Figure 3). The ability to image a broad range of wavelengths accommodates multiple binding dyes for gel imaging. This feature allows the GelSolo to be versatile in its applications, whether for a quick confirmation of product or more in-depth genomic or proteomic studies.

Gel electrophoresis is a useful diagnostics tool in molecular biology, where nucleic acid fragments can be isolated, and an experiment successfully continued. By comparing sized bands from the simulated ladder on the left side of each gel in Figure 3, the sizes of the generated bands from the sample to be analyzed can be determined. Fragments can also be excised from the gel and purified for further examination and manipulation, common in molecular cloning.

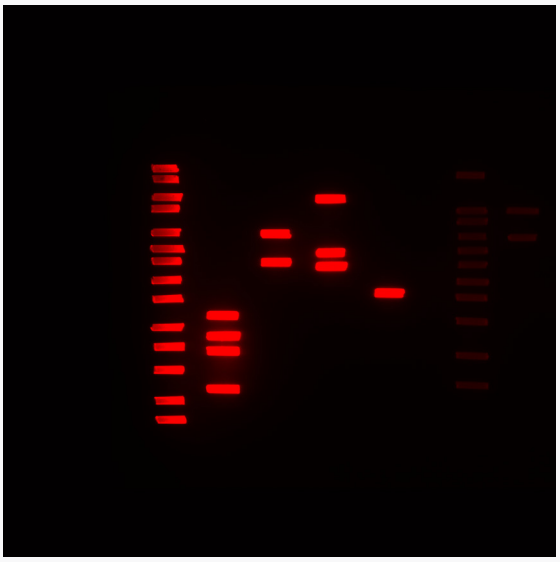

Figure 3. a) Silicone gel image with red pseudocolor representing GelRed bound DNA fragments.

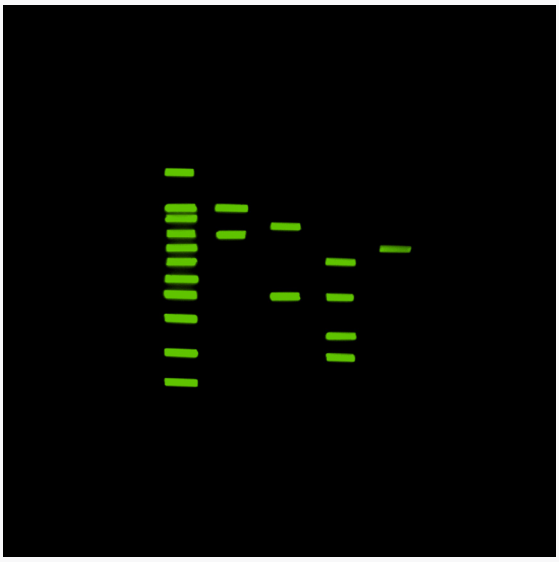

Figure 3. b) Silicone gel with green pseudocolor representing GelGreen bound DNA fragments

References

1. Gallagher, SR and Wiley, EA. Current Protocols: Essential Laboratory Techniques. 2008.

2. Armstrong, J and Schulz, J. 2008. Agarose Gel Electrophoresis. Curr. Protoc. Essential Lab. Tech. Unit 7.2

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