

IMAGING

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Imaging Fluorescently Stained DNA with Alternatives to Ethidium Bromide Using the XcitaBlue[™] Conversion Screen

Introduction

Ethidium bromide (EtBr) is the most commonly used fluorophore for staining DNA due to its availability and low cost. However, it is a powerful mutagen and requires special waste disposal procedures. Furthermore, EtBr is optimally excited by UV light (Figure 1), which is known to damage DNA via thymine dimer formation and strand breaks. This in turn leads to decreased efficiency in cloning and transformation (Paabo et al. 1990, Grundemann and Schomig 1996). Therefore, it is desirable to develop the use of nonnoxious and environmentally friendly technologies.

The recently developed GelGreen (Biotium, Inc.) and SYBR[®] Safe (Invitrogen Corp.) stains exhibit low mutagenicity and no toxicity as reported by their manufacturers (refer to safety information on manufacturer's Web sites). These stains absorb optimally in the blue region of the spectrum (410–510 nm), emit in the green region (see Figure 1), and do not require DNA-damaging UV excitation. They have sensitivity equal to or greater than that of EtBr, although they may require slightly longer exposure times. Both stains can be incorporated into agarose gels before electrophoresis or by buffer exchange after electrophoresis.

To use these stains effectively, Bio-Rad developed the XcitaBlue conversion screen. The screen can be fitted in most Bio-Rad imagers and provides an excitation source for GelGreen or SYBR[®] Safe stains by converting an existing UV transilluminator into a blue light emitter with an

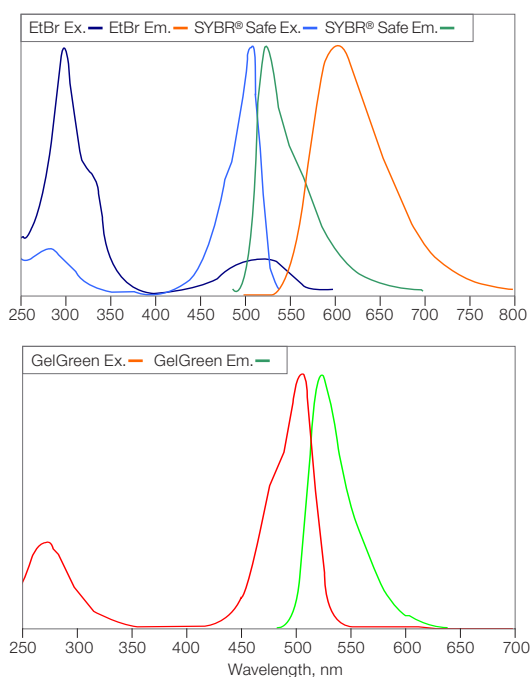


Fig. 1. Excitation (Ex.) and emission (Em.) spectra of ethidium bromide, and SYBR[®] Safe stain (top panel) and GelGreen stain (bottom panel).

optimized excitation peak of ~470 nm. It produces even illumination across the screen and blocks UV light from reaching the gel.

This study describes how using GelGreen and SYBR[®] Safe staining with the XcitaBlue conversion screen and a CCD imager provides equal or better sensitivity than that of EtBr staining and UV excitation.

BIO-RAD

Methods

Serial dilutions of the precision molecular mass ruler (Bio-Rad) were run on 3% TBE agarose gels cast without adding the dyes at 100 V for 45 min. Three gels were run for each experiment and then stained with EtBr (Bio-Rad), SYBR® Safe or GelGreen according to manufacturers' directions. The gels were imaged on a Molecular Imager ChemiDoc XRS+ system using UV transillumination and the standard amber filter (580/120) for EtBr stained gels. The XcitaBlue conversion screen or trans-UV in combination with the 560/50 emission filter were used for SYBR® Safe and GelGreen stained gels. A gel alignment frame (Bio-Rad) was placed on the blue conversion screen or on the UV transilluminator to center the gel. Use of this frame will slightly increase the time to reach saturation, but it also improves signal-to-background ratios, since it blocks excitation light from outside the sample area.

UV light intensity was measured with a UVX digital radiometer (UVP, LLC) with a UVX-31 probe set to the appropriate scale after 2 min of warm-up. Measurements were taken at the center of the transilluminator plate with and without the XcitaBlue conversion screen.

Spectral characteristics of the Xcita Blue screen were measured using an Ocean Optics model S2000 spectrometer. The screen was illuminated by the UV transilluminator on a ChemiDoc XRS+ system to generate the measured spectrum.

Results

Excitation Characteristics

The XcitaBlue screen excitation characteristics are shown in Table 1. The peak excitation wavelength is at 468 nm (Figure 2); it was also shown to pass no UV wavelengths on a transilluminator emitting 7.15 mW/cm².

Table 1. Peak excitation characteristics and UV emission of the XcitaBlue screen.

Peak wavelength	468 nm
UV emission from transilluminator	7.15 mW/cm ²
UV emission from XcitaBlue screen	0 mW/cm ²

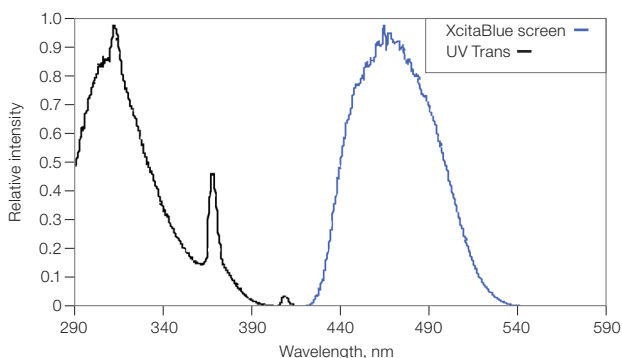


Fig. 2. Emission spectra of UV transilluminator and XcitaBlue conversion screen.

Limit of Detection

The limit of detection for DNA was established by imaging a serial dilution of the precision molecular mass ruler on an agarose gel stained with EtBr using a CCD system, 302 nm UV illumination, and an amber filter. Under these conditions, the lowest visually detectable quantity of the 1 kb DNA band, by adjusting the image using Quantity One® 1-D analysis software, was 0.1 ng (Figure 3, panel A; Table 2).

A slightly higher limit of detection of 0.4 ng was obtained when imaging a SYBR® Safe stained gel using UV excitation and the 560/50 filter, while a limit of detection of 0.1 ng of the 1 kb DNA fragment was reached with GelGreen stain (Figure 3, panels B and C; Table 2) under the same conditions.

When the same gels stained with EtBr, SYBR® Safe, and GelGreen stains were imaged using the XcitaBlue conversion screen and the 560/50 filter, the limits of detection of the 1 kb DNA fragment were 1.6 ng, 0.05 ng, and 0.05 ng (Figure 3, panels D, E, and F; Table 2).

Exposure times when using the XcitaBlue conversion screen are several seconds longer than those for UV light excitation.

Table 2. Limit of detection of DNA on agarose gels.

Stain	Excitation Source	Emission Filter	Integration Time, sec	Limit of Detection of 1,000 bp DNA Band
EtBr	UV	580/120	0.6	0.1 ng
SYBR® Safe	UV	560/50	1.7	0.4 ng
GelGreen	UV	560/50	1.3	0.1 ng
EtBr	XcitaBlue screen	560/50	16.0	1.6 ng
SYBR® Safe	XcitaBlue screen	560/50	10.0	0.05 ng
GelGreen	XcitaBlue screen	560/50	5.0	0.05 ng

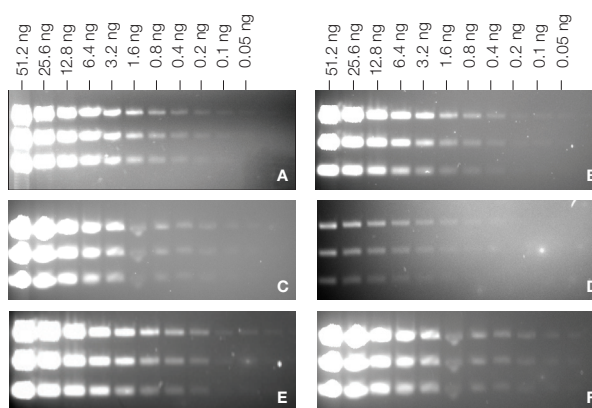


Fig. 3. Limits of detection DNA using different fluorescent stains and illumination wavelengths. A serial two-fold dilution of the precision molecular mass ruler dilution was run on 3% agarose gels. Gels were imaged with the ChemiDoc XRS+ imaging system and Quantity One software, using the following combinations of stain and illumination condition:

- A.** Ethidium bromide, 302 nm UV excitation, 580/120 filter
- B.** SYBR Safe, 302 nm UV excitation, 560/50 filter
- C.** GelGreen, 302 nm UV excitation, 560/50 filter
- D.** Ethidium bromide, XcitaBlue screen, 560/50 filter
- E.** SYBR Safe, XcitaBlue screen, 560/50 filter
- F.** GelGreen, XcitaBlue screen, 560/50 filter

The quantities of the top band (1,000 bp) loaded on the gel were: 51.2, 25.6, 12.8, 6.4, 3.2, 1.6, 0.8, 0.4, 0.2, 0.1, and 0.05 ng.

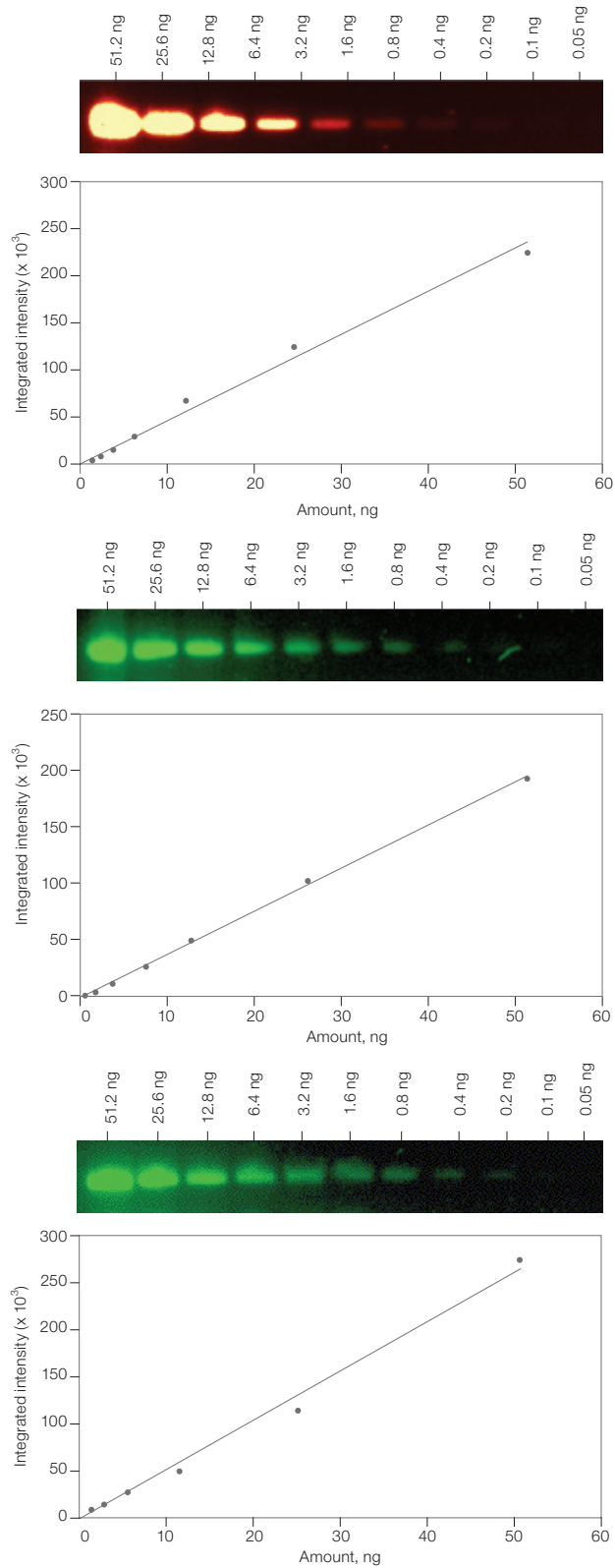


Fig. 4. Quantitation of DNA fluorescence signal on UV illuminated gels. The fluorescence signals of EtBr (top panel), SYBR® Safe (middle panel), and GelGreen (bottom panel) stained DNA fragments were quantitated using the ChemiDoc XRS+ imaging system. Gels were illuminated using a 302 nm transilluminator and a 580/120 or 560/50 filter. Image of gel is shown above corresponding graph.

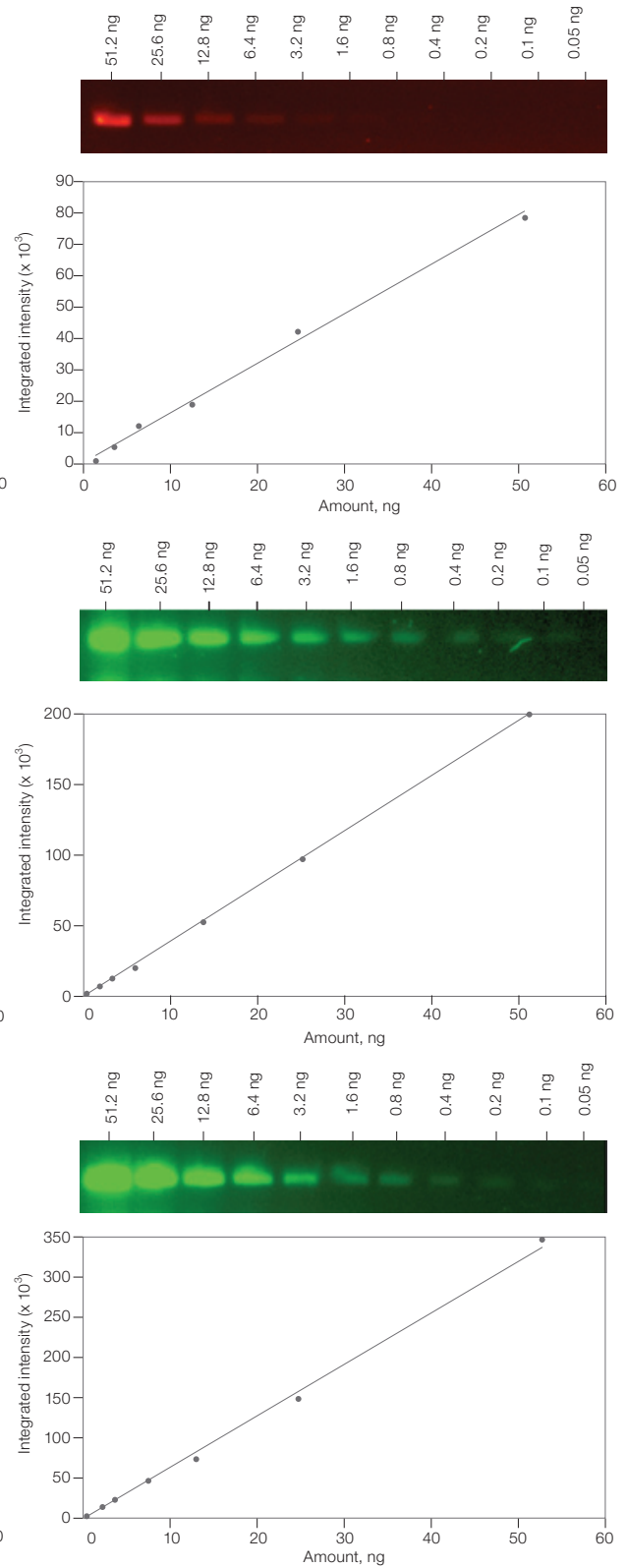


Fig. 5. Quantitation of DNA fluorescence signal on gels illuminated using the XcitaBlue screen. The fluorescence signals of EtBr (top panel), SYBR® Safe (middle panel), and GelGreen (bottom panel) stained DNA fragments were quantitated using the ChemiDoc XRS+ imaging system. Gels were illuminated using a 560/50 filter. Image of gel is shown above corresponding graph.

Linearity

Linearity of fluorescence signal intensity and DNA quantity was measured using a serial dilution of the precision molecular mass ruler run on agarose gels as described. Images were acquired on the ChemiDoc XRS+ imaging system with flat fielding applied. Quantitative analysis using the volume tools in Quantity One software showed that there is a high degree of linearity between the amount of DNA on the gel and the fluorescence signal intensity (Figures 4 and 5, Table 3).

Table 3. Linearity between signal intensity and DNA quantity.

Stain	Excitation Source	Emission Filter	Linearity (R ²)
EtBr	UV	580/120	0.9907
SYBR® Safe	UV	560/50	0.9992
GelGreen	UV	560/50	0.9886
EtBr	XcitaBlue screen	560/50	0.9948
SYBR® Safe	XcitaBlue screen	560/50	0.9992
GelGreen	XcitaBlue screen	560/50	0.9937

Conclusion

The XcitaBlue conversion screen makes use of nontoxic dyes convenient for visualizing nucleic acids on agarose gels. Results display high sensitivity and can be obtained without harmful UV exposure.

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