Fluorescent DNA Gel Stain Detection

Typhoon Variable Mode Imager

Key words: fluorescence, imaging, DNA gels, Typhoon, ethidium bromide, Vistra Green

Fluorescence detection of DNA gels is used in molecular biology laboratories to visualize the results of DNA preparations, restriction digests, and PCR reactions, as well as other more specialized applications. The fluorescent gel stain ethidium bromide is a popular choice for routine nucleic acid detection. More sensitive stains, such as Vistra Green,™ are available to address a broad range of DNA applications requiring lower detection limits in both agarose and polyacrylamide gel formats.

Typhoon™ Variable Mode Imagers provide sensitive fluorescence detection of common DNA gel stains, including ethidium bromide and Vistra Green. The Typhoon 8600 and 9200 series have two excitation sources for fluorescence imaging: a green (532 nm) and a red (633 nm) laser. The Typhoon 9400 series has an additional blue laser with two excitation lines (457 nm and 488 nm).

Products used

Typhoon 8600	63-0027-96
Typhoon 9200	63-0038-49
Typhoon 9210	63-0038-51
Typhoon 9400	63-0038-53
Typhoon 9410	63-0038-55
Agarose, high efficiency separation	US10132
EPS 301 Power Supply	18-1130-01
Hoefer™ HE 99X Max Submarine Unit	80-6061-38
Hoefer miniVE Vertical Electrophoresis System	80-6418-77
Low Fluorescence Glass Plate, 3-mm-thick	63-0028-92
PlusOne™ reagents	
Acrylamide	17-1302-01
Ammonium Persulfate	17-1311-01
Bromophenol Blue (BPB)	17-1329-01
Ethidium Bromide Solution (10 mg/ml)	17-1328-01
N,N'-Methylene-Bis-Acrylamide	17-1304-01
TEMED	17-1312-01
TBE Buffer, premixed powder 10×	US70454
TE Buffer, 50×	US75834
Vistra Green Nucleic Acid Stain	RPN5786

Other materials required

- Low DNA mass ladder (Invitrogen™)
- 6× sample buffer (40% sucrose in water, bromophenol blue)

Protocol

1

Preparing the gel

1.1. Cast a 1% agarose gel or a 10% non-denaturing polyacrylamide gel in 1× TBE buffer.

1.2. To cast an agarose gel with ethidium bromide, add the dye to the cooled, molten gel solution at 0.5 µg/ml just before pouring.

2

Preparing the sample

2.1. Mix 8 µl of a 1:10 dilution of the DNA ladder stock (117.5 ng/µl) with 2 µl of 6× sample buffer.

2.2. Dilute 5 µl of the DNA solution serially into 5 µl of 1× sample buffer (two-fold serial dilutions).

3

Performing gel electrophoresis

3.1. Load 5 µl of the prepared sample into the wells.

3.2. Perform electrophoresis using the EPS 301 power supply at 100 V. Run the polyacrylamide gel until the tracking dye migrates to the bottom of the gel. Run the agarose gel until the tracking dye migrates one-quarter to one-third of the way down the gel.

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3.3. Stain the gel in a 1:10,000 dilution of Vistra Green in 50 ml of 1× TE buffer (pH 7.5) for 30 min. For ethidium bromide staining, use a final concentration of 0.5 µg/ml in 50 ml of 1× TE buffer (pH 7.5). Stain the gel for 30 min with gentle agitation in a polypropylene container (longer staining times may be needed for high percentage gels). For ethidium bromide, destain the gel for 30 min in deionized water.

4

Imaging the gel

4.1. In general, gels with a thickness less than 2 mm can be imaged on a 3-mm-thick, low-fluorescence glass plate using the +3 mm focal plane setting. The glass plate helps to keep the gel intact permitting future analysis steps. The plate also helps to protect the Typhoon glass platen from temporary contamination by the DNA stains, which is a problem common with these stains on all imagers. A thorough cleaning of the glass plate and the platen is recommended immediately after scanning (see 4.3).

Affix two Kapton™ tape strips along the edges, one on each long side of the low-fluorescence glass plate. Pour sufficient deionized water onto the platen to form a pool. Turn the plate over so that the tape faces downward, and gently place the low-fluorescence glass plate on top of the water to minimize the appearance of interference patterns. Avoid trapping any bubbles between the glass plate and the platen. Pour a small amount of deionized water onto the glass plate. Place the polyacrylamide gel on top of the water. Avoid bubble formation between the gel and the glass plate.

Alternatively, gels less than 2 mm in thickness can be imaged by using "platen" for the focal plane setting and directly placing it on the platen on a small amount of deionized water. Avoid bubble formation between the gel and the platen. In general, gels with a thickness greater than 3 mm can be imaged by placing the gels directly on the platen and use the +3 mm focal plane setting. A thorough cleaning of the platen is recommended immediately after scanning (see 4.3).

4.2. In the Scanner Control Setup window, choose the appropriate laser and emission filter combination as recommended in Table 1. In addition, choose an appropriate PMT voltage setting (450-800 V) and focal plane setting (see 4.1), as well as the following parameters:

- Pixel size: 100 µm
- Sensitivity: Normal

For more details about Typhoon emission filter selection and other practical aspects of using Typhoon imagers, please refer to *Typhoon Instrument Guide* as well as *Fluorescence Imaging: principles and methods* (1).

4.3. After scanning, use a soft, lint-free cloth dampened with deionized water to rinse the glass plate, the platen and, if necessary, the sample lid. Repeat the procedure with 75% ethanol. Rinse again with deionized water to remove any possible fluorescent residue from the ethanol. If the glass plate or the platen is still dirty, clean with a freshly made 5–10% hydrogen peroxide solution followed by extensive rinsing with deionized water to remove residual hydrogen peroxide.

Results

Four images of fluorescently stained DNA gels that were imaged on Typhoon are shown in Figure 1. The top two images are of agarose gels, one cast with ethidium bromide and one stained with Vistra Green. The bottom two images are of polyacrylamide gels, one stained with ethidium bromide and one stained with Vistra Green. Using the experimental conditions described in this application note, similar results were achieved using either the 488 nm or the 532 nm laser line for Vistra Green detection of DNA (Fig 2).

It is important to note that the detection limits of these post-staining gel applications are not limited by the performance of the Typhoon imager. This is strongly supported by the fact that using an excitation wavelength closer to a stain's excitation maximum, or increasing laser power, may not significantly

Gel stain (excitation, emission)	Laser	Emission filter
Ethidium bromide (526 nm, 605 nm)	Green (532 nm)	610 BP 30
Vistra Green (490 nm, 520 nm)	Blue (488 nm)*/Green (532 nm)†	520 BP 40*/526 SP†

*Laser/emission filter combination for the Typhoon 9400 series.
†Laser/emission filter combination for the Typhoon 8600 and 9200 series.

Table 1. Recommended Typhoon Scanner Control settings for the detection of fluorescent DNA gel stains.

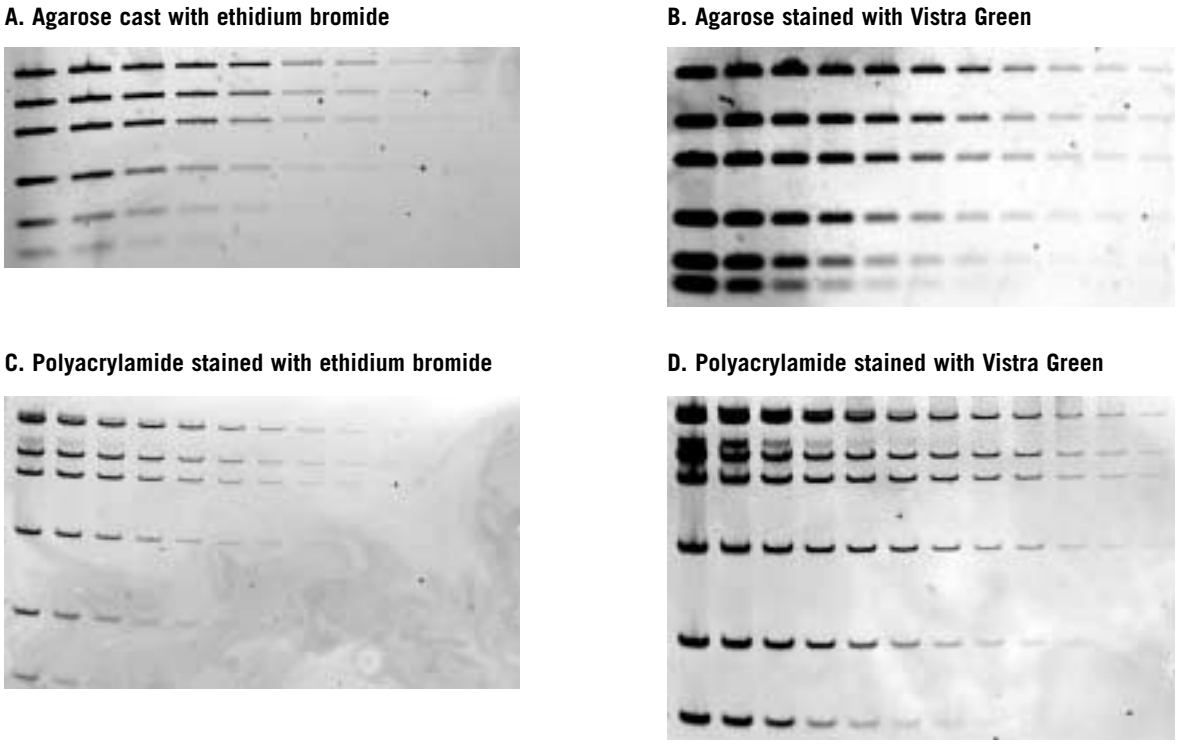

Figure 1. Two-fold serial dilutions of the DNA mass ladder imaged using Typhoon. 1% agarose gels were cast with ethidium bromide (A) or stained with Vistra Green (B). Starting quantities were 20 ng of 2000 bp, 12 ng of 1200 bp, 8 ng of 800 bp, 4 ng of 400 bp, 2 ng of 200 bp, and 1 ng of 100 bp. 10% polyacrylamide gels were stained with ethidium bromide (C) or Vistra Green (D). Starting quantities were 10 ng of 2000 bp, 6 ng of 1000 bp, 4 ng of 800 bp, 2 ng of 400 bp, 1 ng of 200 bp, and 0.5 ng of 100 bp. Gels stained with Vistra Green were imaged at 488 nm.

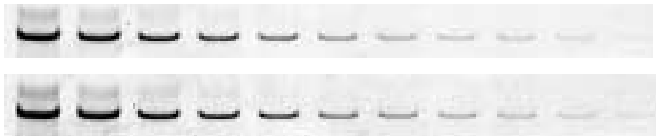

Figure 2. Vistra Green staining of a 1.2 kb DNA band (second band from top of gel) resolved in a 10% polyacrylamide gel and imaged by Typhoon 488 nm (top image) with the 520 BP 40 emission filter and 532 nm (bottom image) laser lines with the 526 SP emission filter. From left to right, lanes 1 to 11 contain 3000 pg, 1500 pg, 750 pg, 375 pg, 188 pg, 94 pg, 47 pg, 24 pg, 12 pg, 6 pg, and 3 pg DNA.

improve the detection limits of these applications. For example, judging from the excitation maximum of Vistra Green at 490 nm, using the 488 nm laser line to excite Vistra Green is, in theory, more "optimal" than using 532 nm. However, the results in this application note demonstrate that 488 nm and 532 nm offer essentially similar imaging results for Vistra Green stained DNA gels (Fig 2). The major factors limiting detection are the background staining of the gel, the imperfect stain/DNA binding stoichiometry, and other experimental factors.

Table 2 summarizes the limit of detection (LOD) and linear ranges for ethidium bromide and Vistra Green stained DNA gels using the Typhoon instrument settings listed in Table 1. LOD is the threshold at which the background-corrected signal-to-noise ratio is at least 3. Similar LOD and linear range values are obtained when using SYBR™ DNA gel stains with Typhoon (results not shown). Note that Typhoon offers a wide linear dynamic range of five orders of magnitude (from count 1 to 100 000). Nevertheless, the linear range of DNA

Gel stain	LOD (pg/band)	Linear range (fold)
Ethidium bromide (cast with gel)	50/ND*†	~500/ND
Ethidium bromide (post-stain)	50/6	~500/1000
Vistra Green-post-stain	10/3	~500/1000

*First number for 1% agarose gel/Second number for 10% polyacrylamide gel
†ND = Not Determined

Table 2. Typhoon LOD for the 1.2 kb DNA band in either 1% agarose or 10% polyacrylamide gels.

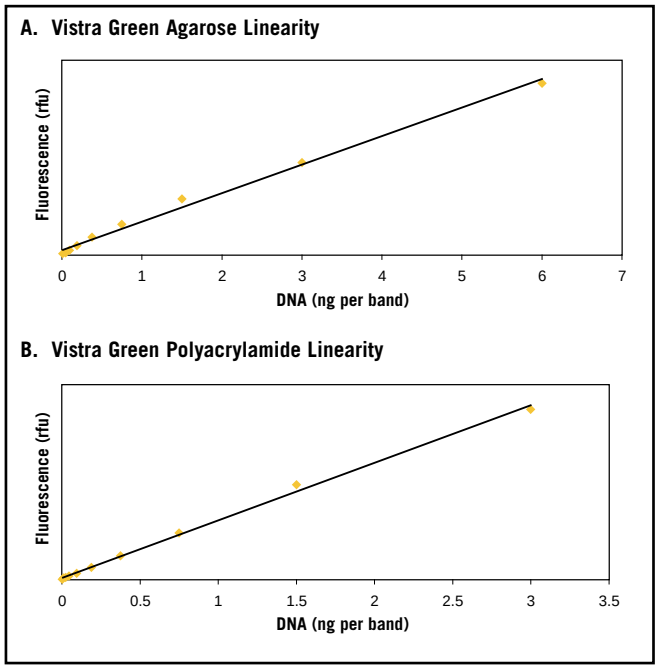

Figure 3. Fluorescence intensity of 1.2 kb DNA bands stained with Vistra Green versus DNA amount (ng per band) from an agarose gel (A, from 12 pg to 6 ng) and a polyacrylamide gel (B, from 3 pg to 3 ng) imaged with the 532 nm laser line. Fluorescence refers to integrated relative fluorescence units (rfu) from ImageQuant™ volume analysis using rectangle objects and histogram peak background correction. The linear fit to data has an R^2 value of at least 0.99.

detection is typically limited to two or three orders of magnitude (Fig 3) by a variety of experimental factors mentioned in the previous paragraph. This suggests that Typhoon has the potential to offer even better imaging results when improved fluorescent staining methods become available.

Conclusion

The Typhoon imaging system provides sensitive digital imaging and quantitative analysis of common DNA fluorescent gel stains. The Typhoon limits of detection for DNA in both agarose and polyacrylamide gels are equal to or better than sensitivities reported using standard ultraviolet illumination and camera systems for image capture (2, 3). The laser lines, in combination with high quality optics, are suitable for the detection of ethidium bromide and Vistra Green DNA stains. The linear quantitative range for the fluorescent DNA gel stains, in both gel systems, is from 2.5 to 3 orders of magnitude. The selectable focal plane available with Typhoon allows detection of samples of variable thickness and permits optimal image acquisition from a variety of electrophoresis gel formats.

References

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3. Tuma, R. S. *et al., Anal. Biochem.* 268, 278–288 (1999).

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