Fluorescent gel imaging with Typhoon 8600

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GEL AND BLOT IMAGING

Typhoon™ 8600 variable mode imager is capable of fluorescence, storage phosphor, and chemiluminescence detection of gels and blots. This article highlights several fluorescence applications. For DNA, as little as 10 pg of DNA per band (1 kb) in a polyacrylamide gel could be detected using Vistra Green™ gel stain✧. For protein, the limit was 2 ng per band of BSA using SYPRO™ Orange or Red gel stains. The ability of Typhoon 8600 to separate multi-colour fluorescent signals was demonstrated in DNA fragment analysis and electromobility shift assay applications. Use in differential display analysis was also demonstrated.

Introduction

Fluorescence detection is used in the molecular biology research laboratory for applications such as gel documentation, quantification of nucleic acids, microscopy, and expression analysis. Sensitive fluorescent gel stains are available for both nucleic acid and protein detection. In other gel applications, detection of specific target molecules is accomplished using distinct fluorescent tags, such as Cy™5.

When labelling target molecules, the use of multiple fluorescent tags enables discrimination between different classes of molecules in complex mixtures. Fluorescent multiplexing relies on optimal excitation and collection of fluorescent emission from each label. Using this approach, the efficiency and accuracy of analysis is maximized.

Multi-label analyses require a sensitive detection system capable of resolving multiple fluorescent signals. Typhoon 8600 is a versatile, variable mode imager capable of fluorescence, storage phosphor, and chemiluminescence detection of gels and blots. The system uses two lasers—green (532 nm) and red (633 nm)—together with highly sensitive optics for detection of one to four different fluorescent labels. Selectable optical focal depth allows efficient imaging of a variety of sample formats including agarose gels, polyacrylamide gels (free or sandwiched between glass plates), membranes and microplates.

Here we present a survey of the fluorescent gel applications that can be performed using Typhoon 8600.

General nucleic acid gel stains

The DNA gel stains ethidium bromide and Vistra Green were evaluated for use with Typhoon 8600. Serial dilutions of a DNA molecular weight ladder were separated by agarose and polyacrylamide gel electrophoresis and the gels stained with either dye. Using Typhoon, the limit of detection for ethidium bromide stained agarose gels is at least 60 pg per band of DNA (Table 1). Vistra Green, a more sensitive DNA gel stain, detects as little as 10 pg per band in a polyacrylamide gel.

Table 1. Typhoon 8600 limit of detection for 1 kb DNA band in either 1% agarose or 10% polyacrylamide gels

	limit of detection* (1 kb band)	
DNA gel stain	**agarose gel (pg)**	**polyacrylamide gel (pg)**
ethidium bromide—cast with gel	60	-
ethidium bromide—post stain	50	30
Vistra Green—post stain	25	10

** Limit of detection is the threshold at which peak band signal (background-corrected) over noise is greater than 3.0. All measurements determined with ImageQuant™ image analysis software.*

Protein gel stains

The fluorescent protein gel stains SYPRO Orange, SYPRO Red, and SYPRO Ruby were tested using bovine serum albumin separated in a Tris-glycine polyacrylamide gel. Results from SYPRO Red are shown in Figures 1 and 2. The limits of detection for the three SYPRO protein gel stains are summarized in Table 2.

Table 2. Limit of detection for three SYPRO gel stains

SYPRO gel stain	limit of detection* (ng BSA per band)
Orange	2
Red	2
Ruby	4

** Limit of detection is the threshold at which peak band signal (background-corrected) over noise is greater than 3.0. All measurements determined with ImageQuant image analysis software.*

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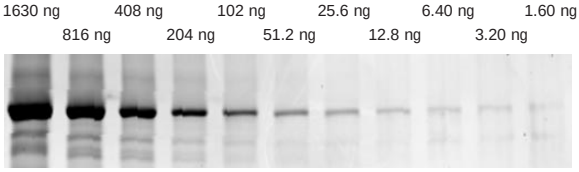

Fig 1. SYPRO Red detection of bovine serum albumin in a Tris-glycine gel. 532 nm excitation was used with a 610BP30 emission filter. Two-fold serial dilutions of BSA were made to give a dilution series from 1.6–1630 ng.

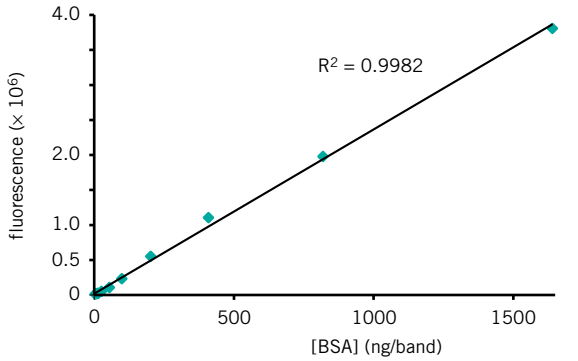

Fig 2. Fluorescence intensity of BSA bands from Figure 1. Fluorescence refers to integrated relative fluorescence units (rfu) from ImageQuant volume analysis using rectangle objects and local median background correction. Linear fit to data has R^2 value of greater than 0.99.

Multi-colour fluorescent gel applications

DNA gel assays frequently require quantitative characterization of multiple fragments based on size or amount. Both experimental accuracy and efficiency can be improved by the use of multiple fluorescent labels to define different classes of fragments in the same gel. For example, size analysis of unknown fragments is accomplished most accurately when those fragments are resolved in the same gel lane as the standards.

Many current DNA-based assays use PCR✧ to specifically amplify one or more desired DNA targets from small amounts of starting material. Since PCR primers can be individually tagged with different fluorescent labels, these assays are ideally suited for multi-channel fluorescence design.

DNA fragment analysis

To demonstrate the multi-channel capability of Typhoon 8600, multiple fluorescently tagged DNA fragments were resolved in the same gel and combined in the same gel lanes. A mixture of size ladders and PCR products was used to generate the image shown in Figure 3. The combined ability of Typhoon 8600 optics and FluorSep™ software to spectrally separate different labels is illustrated in Figures 3B and 3C.

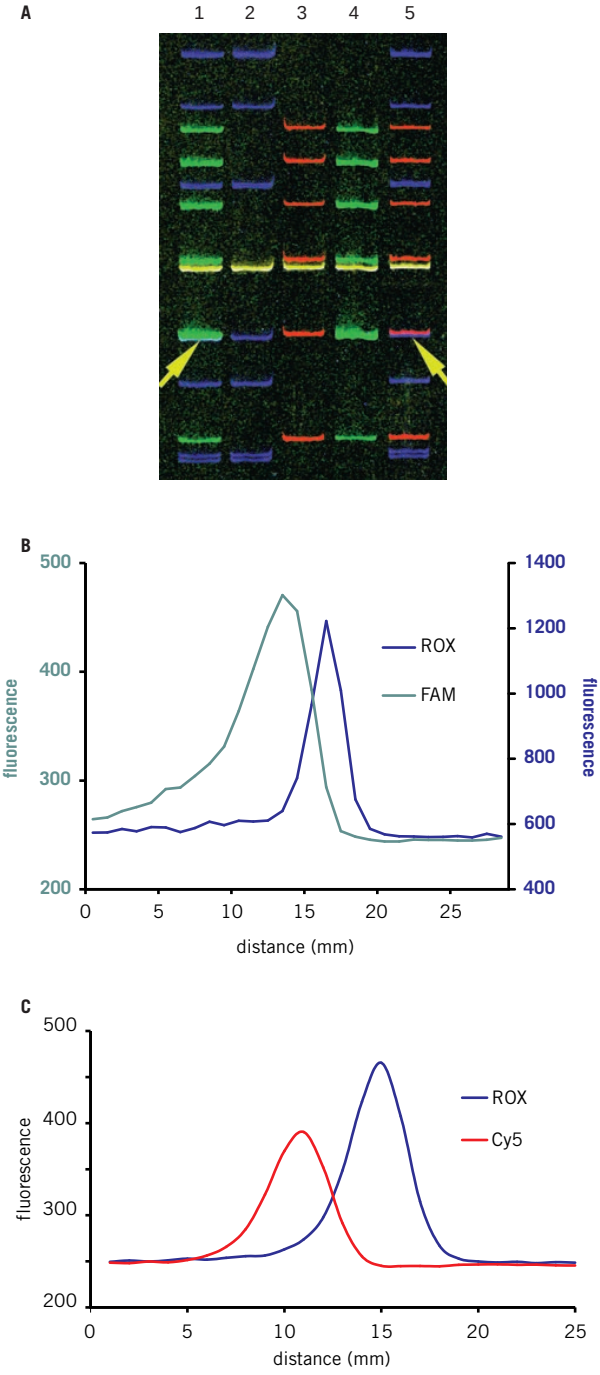

Fig 3. (A) Four-colour fluorescent DNA gel. Fluorescein Sizer 50–500 (green), Cy3-labelled PCR product (yellow), ROX™ GS500 (blue, Perkin Elmer) and ALFexpress™ Cy5 Sizer 50–-500 (red) were resolved in a 10% polyacrylamide gel at 2– 5 fmol per band. 532 nm excitation was used with 526SP, 580BP30 and 610BP30 emission filters. 633 nm excitation was used with a 670BP30 emission filter. FluorSep software was used to minimize fluorescent overlap between labels in the four different channels. (B) Resolution between two sets of closely migrating bands (arrows) in lane 1 and lane 5 (C) is shown. Fluorescence refers to integrated relative fluorescence units (rfu) determined using ImageQuant. Distance refers to distance through the bands in each of the two channels.

✧ *See licensing information on page 3.*

Figure 4 shows analysis of genomic DNA inserts from bacterial artificial chromosome (BAC) and cosmid clones using three different fluorescent labels. Due to the large size of DNA fragments, electrophoresis was performed using an agarose gel. The gel was imaged directly, without drying or other processing.

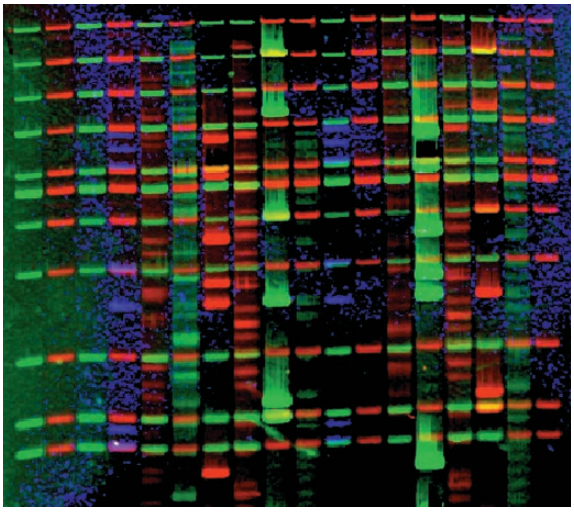

Fig 4. BAC and cosmid genomic clone sizing using three-colour labelling. FAM (blue), HEX (green) and ROX (red) labels were used. Samples were resolved in a 1% agarose gel and imaged using 532 nm excitation with 526SP, 580BP30 and 610BP30 emission filters. (Courtesy of Laurie Gordon, Lawrence Livermore National Laboratory, Livermore, CA, USA.)

Multi-colour DNA-protein electromobility shift assay

Fluorescent gel shift assays are rapid and sensitive alternatives to traditional detection using radioactivity. A multi-colour gel shift experiment is shown in Figure 5. The Mnt repressor protein was bound to various DNA oligonucleotide targets end-labelled with different fluorochrome labels. Using Typhoon 8600 multi-channel imaging, this approach permits discrimination of multiple DNA-protein complexes produced in the same binding reaction.

Expression analysis

Differential display analysis (DDA) enables direct side-by-side comparison of complex expression patterns from many samples. Following PCR amplification, fragments are separated on high-resolution denaturing polyacrylamide gels, from which bands of interest can be isolated and subjected to further analysis. Due to its relative simplicity and sensitivity, DDA has gained wide acceptance as a gene expression research tool.

A Cy5-based DDA is shown in Figure 6. A band representing differential expression between the two different tissue types was isolated and PCR amplified for further analysis.

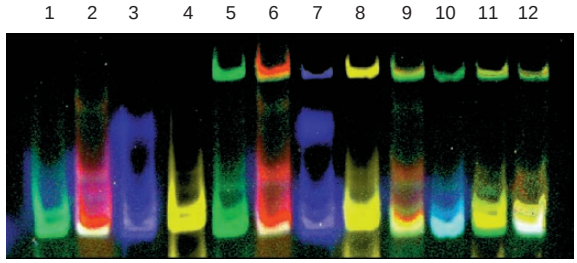

Fig 5. Multi-label gel shift experiment. 532 nm excitation was used with 526SP, 555BP30, 580BP30 and 610BP30 emission filters. The first four lanes (left to right) show 0.4 pmol of four different labelled 180 bp DNA fragments: FAM™ (green), HEX™ (red), TAMRA™ (blue) and ROX (yellow). Lanes 5–8 are the same four labelled DNA fragments mixed with Mnt protein—the bound DNA complexes migrate as larger shifted bands. Lanes 9–12 have mixtures of bound labelled fragments: lane 9 (FAM + HEX), lane 10 (FAM + TAMRA), lane 11 (FAM + ROX) and lane 12 (FAM + HEX + TAMRA + ROX). (Samples courtesy of Chris Man, Washington University School of Medicine, Department of Genetics, St. Louis, MO, USA.)

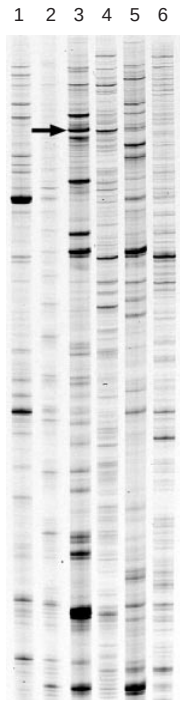

Fig 6. Differential display analysis using Cy5 detection. Reactions were carried out on mRNA from lung and liver tissue. The six lanes are grouped into three pairs comparing lung and liver expression (lung is the even numbered lane of each pair). The Cy5 version of a displayPROFILE™ kit from Display Systems Biotech was used. The denaturing polyacrylamide sandwich gel was imaged directly using 633 nm excitation with a 670BP30 emission filter. Arrow indicates the band that was excised for further sequence analysis.

Conclusion

Fluorescence is a safe, sensitive, quantitative, and versatile labelling method. The ability to detect and resolve multiple fluorochromes in the same sample improves experimental efficiency and accuracy. The growing availability of fluorescence-based kits and sensitive detection equipment, such as Typhoon 8600, eases the transition from radioactivity to fluorescence protocols.

ORDERING INFORMATION

Typhoon 8600	1	8600PC