

SCIENCE IMAGING SYSTEMS

# Application Note

**No. 3**

DNA Detection with EtBr

FLA-2000

## Introduction

Ethidium bromide (EtBr) is a fluorescence reagent widely used in genomic analysis. Since, like SYBR® Green I (Application Note No.2), EtBr fluoresces when intercalated into DNA, it can be used for direct staining of DNA in gel electrophoresis. It is an inexpensive reagent readily available from many different manufacturers. EtBr stained DNA can be detected with the FLA-2000 using the 473 nm laser light source.

As one disadvantage of using EtBr is the high background of the image, the FLA-2000 is equipped with two filters (520nm and 580nm) which substantially eliminate the background of the gel itself. By proper use of these filters, a low-background image can be obtained with high efficiency.

This Application Note outlines the basic characteristics of EtBr and explains the image flip function of the MacBAS software.

The editors are welcome to receive your comments and suggestions on using the FLA-2000.

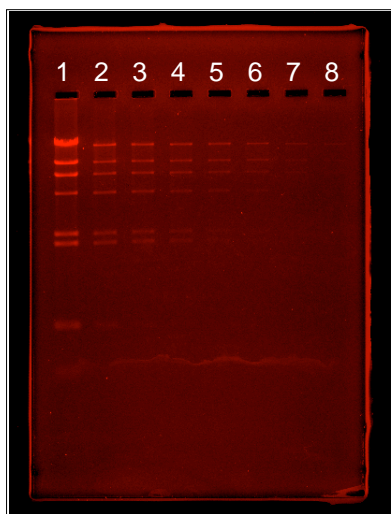
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## Summary

- Images with low background noise can be obtained by using the O580 read-out filter to cut wavelengths under 580nm.
- Detection to about 194pg was achieved in a test using EtBr.

# 1 Basic EtBr Data



**Explanation of image sample**

Sample :  $\lambda$ DNA/*Hind* III  
 Applied amount :

- Lane-1 : 1mg
- Lane-2 : 100ng
- Lane-3 : 50ng
- Lane-4 : 20ng
- Lane-5 : 10ng
- Lane-6 : 5ng
- Lane-7 : 2ng
- Lane-8 : 1ng

The applied sample was electrophoresed at 50V constant voltage.

**FLA-2000 read-out conditions**

- Gradation : 65536(16bit)
- Resolution : 50mm
- Sensitivity : F10
- Latitude : 5
- Sample Mode : Fluor.473nm  
: O580Filter

## Reagent Properties

|                |  |
|----------------|--|
| Name           | EtBr (ethidium bromide)  |
| Classification | Fluorescence dye (intercalating type)  |
| Nature         | Emits red fluorescence in the presence of DNA and RNA.   |
| Sample         | dsDNA, ssDNA, RNA  |
| Storage        | Keep in dark freezer. The aqueous solution should ordinarily be prepared at a concentration of 10mg/ml.                          |
| Caution        | Since intercalation of the reagent into DNA is possible, there is a potential hazard of mutagenicity. Wear gloves when handling. |
| Manufacturer   | (Various)  |

### Processing waste EtBr for disposal.

EtBr is known to be mutagenic. When handling it, wear gloves and other protective clothing and take measures to minimize release into the laboratory environment. Gels and waste solution should be treated with sodium hypochlorite or activated charcoal. Methods for conducting these treatments can be found in many laboratory handbooks.

## Excitation/Emission Spectra of EtBr

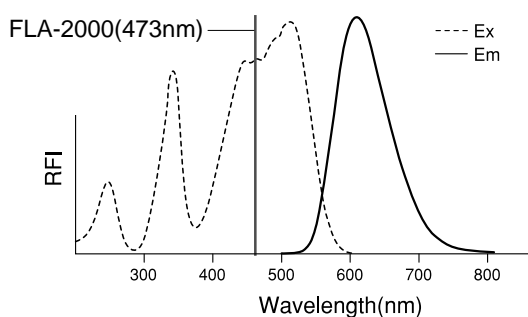


Fig.1-1

### RFI

Relative fluorescence intensity

### Fig.1-1 Excitation/Emission Spectra of EtBr stained DNA

## DNA Amount vs. Fluorescence Intensity

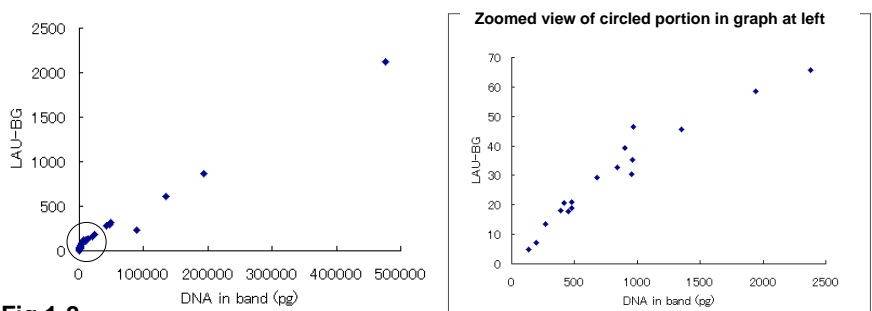


Fig.1-2

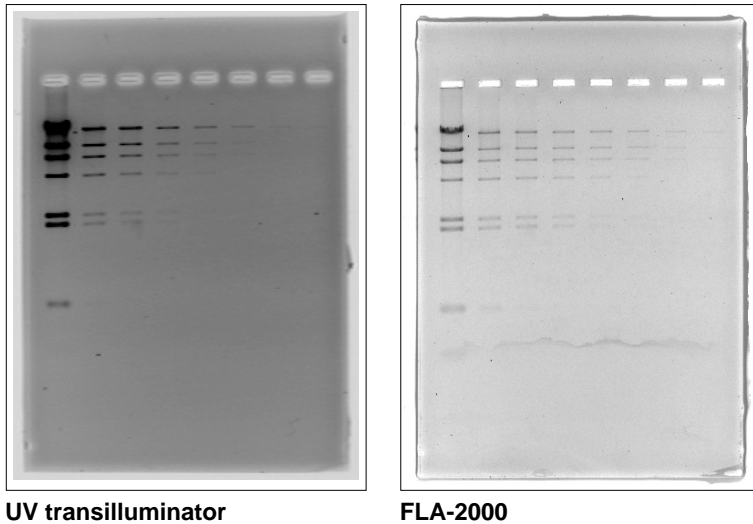
### Fig.1-2 Quantitative Fluorescence Response of EtBr Stained Electrophoresis Gel of $\lambda$ DNA/*Hind* III

Left : Amount of DNA vs. Fluorescence Intensity  
 Right : Zoomed view of circled portion of graph at left.  
 The data was obtained using the profile analysis feature of the MacBAS software for the FLA-2000. Background was subtracted.  
 (LAU: Linear Arbitrary Unit is the fluorescence intensity unit used in the FLA-2000.)

## 2 DNA Detection Sensitivity Using EtBr

The detection limit of EtBr stained DNA using an UV transilluminator is generally accepted to be approximately 1ng. With the FLA-2000, detection to 194pg was possible.

The data obtained for the same sample using an UV transilluminator (312nm excitation) and the FLA-2000 (473nm excitation/580nm filter) are compared below.



UV transilluminator  
Fig.2-1

FLA-2000

**Fig.2-1 FLA-2000 and UV Transilluminator Comparison**

Left : Image by  
UV transilluminator  
Right : Image by FLA-2000

Electrophoresis was carried out on  $\lambda$ DNA/*Hind* III applied to Lanes 1-8 in amounts of 1 $\mu$ g, 100ng, 50ng, 20ng, 10ng, 5ng, 2ng, and 1ng. Staining was conducted in a 1 $\mu$ g / ml solution of EtBr for 2 hours.

## 3 Effect of Using Filters

The FLA-2000 permits selection of either a Y520 or an O580 filter during read-out using the 473nm SHG laser. Since the filters cut wavelengths under 520 nm and 580nm, respectively, they can be used to eliminate or reduce the noise component and enhance image sensitivity.

As shown in the graph below, the gel is itself a source of background noise. When EtBr is used, more effective background noise reduction can be obtained with the O580 nm filter than with the Y520 filter.

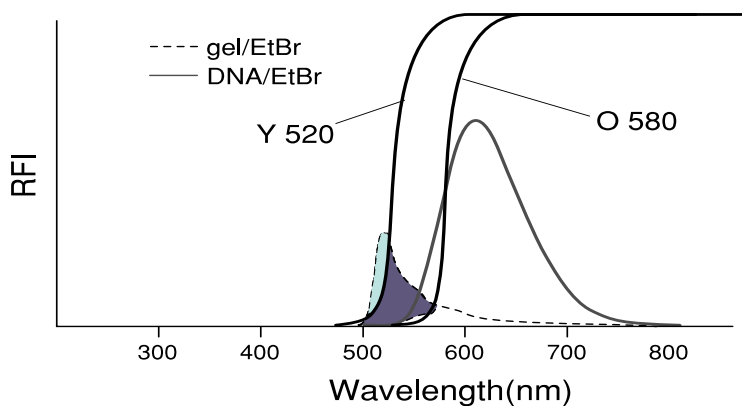


Fig.2-2

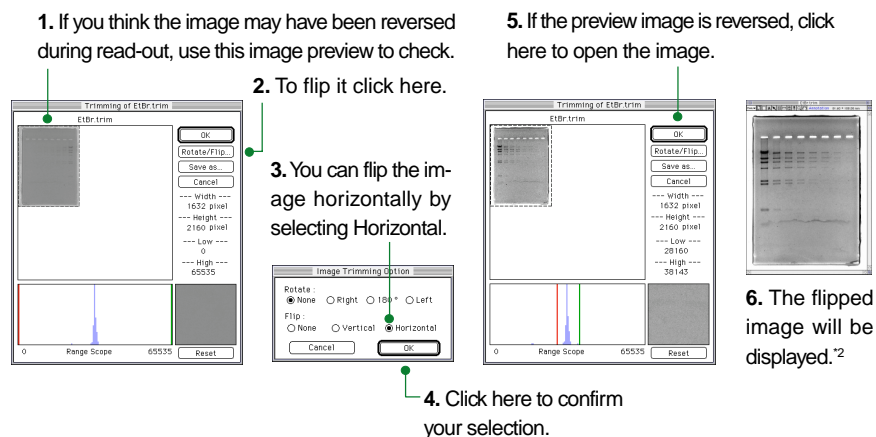
**Fig.2-2 How filters cut gel noise but pass the fluorescence of EtBr stained DNA**

The transmittance of Y520, and O580 filter are shown along with fluorescence spectrum of gel and DNA both stained by EtBr

## 4 One-Point Advice on Analysis Using MacBAS

Q.2 I made a mistake when I placed an EtBr sample on the FLUOR stage of the FLA-2000 for read-out and got a reversed image. Is there any way to correct for this?

A.2 If the read-out image is reversed when you open it in MacBAS, you can easily flip it over\*1. Confirm that the image has been reversed in the Trimming of.... dialog and then click on the OK button.



### Flow of the analysis

- **Open image**
- Adjust image for easier viewing
- Quantify
- Print
- Use data with other software
- **Save data**
- Terminate

\*1 An image that has already been saved can also be flipped by selecting it in File - Trim Again... and then following the procedure explained here.

\*2 The flipped image is not automatically saved at this point. To save it, use the File - Save.. feature. You can also save it at the time you close the image by answering Yes to "Save this file before closing?"

## 5 References

1. Waring, M.J. Complex formation between ethidium bromide and nucleic acids, *J. Mol. Biol.* 13:269-282 (1965).
2. Sharp, P. A., Sugden, B., Sambrook, J. Detection of two restriction endonuclease activities in *Haemophilus parainfluenzae* using agarose-ethidium bromide electrophoresis, *Biochemistry* 12:3055-3063 (1973).
3. Lunn, G., Sansone, E. B. Ethidium bromide: Destruction and decontamination of solutions, *Anal. Biochem.* 162:453-458 (1987).
4. Menozzi, F. D., Michel, A., Pora, H., Miller, A. O. A. Absorption method for rapid decontamination of solutions of ethidium bromide and propidium iodide, *Chromatographia* 29:167-169 (1990).
5. Grundemann, D., Koepsell, H. Ethidium bromide staining during denaturation with glyoxal for sensitive detection of RNA in agarose gel electrophoresis, *Anal. Biochem.* 216:459-461 (1994).
6. Dutton, M. D., Varhol, R. J., Dixon, D. G. Technical consideration for the use of ethidium bromide in the quantitative analysis of nucleic acids, *Anal. Biochem.* 230, 353-355 (1995).

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