

3 Staining Time vs. Fluorescence Intensity

Staining can be effected by the method of adding the SYBR® Green I to the gel or to electrophoresis buffer in advance (see Reference I.) or by the method of shaking the electrophoresed gel in a SYBR® Green I staining solution (see References 1., 2. and 3.).

To obtain an index of the staining time required by the shaking method, the relationship between staining time and fluorescence intensity was investigated for a 5mm-thick 1% agarose gel sample. The results below show the required shaking time to be 30-60 minutes, approximately the same as in the conventional (EtBr) method.

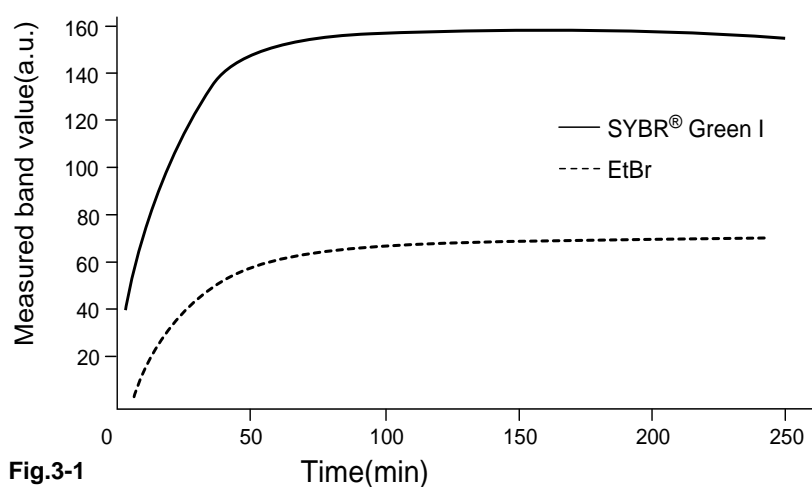


Fig.3-1

a.u.
Arbitrary unit

Fig.3-1 Staining Time vs. Fluorescence Intensity for SYBR® Green I and EtBr

■ Staining Conditions in the References

The staining times in the references that use SYBR® Green I are shown in the table below. In all cases, SYBR® Green I was used to detect DNA after PCR.

Gel	Staining solution time	Staining time	Reference
3% Nu Sieve agarose 1% GTG agarose	1:10,000 / 1 x TAE	25min	1.
10% polyacrylamide 17cmx13.5cmx1.5mm	1:10,000 / 50mM Tris-HCl	45min	2.
7.5% polyacrylamide	1:20,000	5-20min	3.

PCR (Polymerase Chain Reaction)

A method used to produce a large amount of a specific DNA fragment. Heat-resistant DNA polymerase is used for DNA replication. Thermal cycling (high → low → high) of dsDNA through ssDNA is repeated to double the amount of the specific DNA fragment with each cycle.

4 DNA Detection Sensitivity Using SYBR® Green I

■ FLA-2000 and UV Transilluminator Comparison

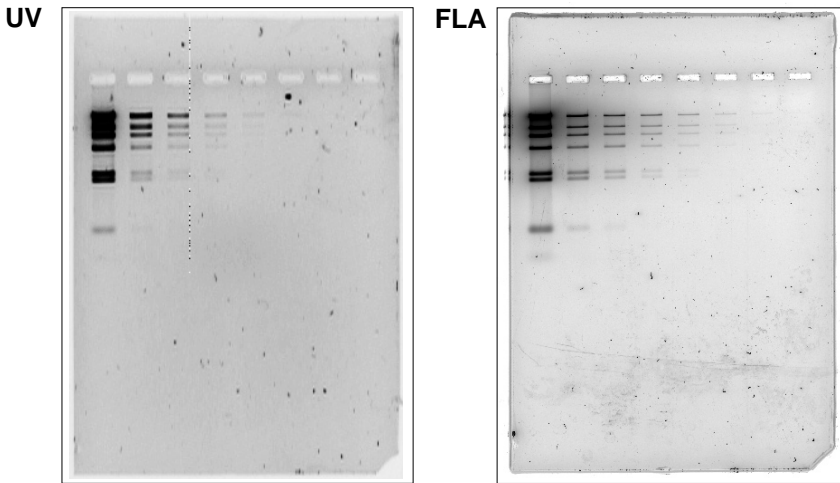


Fig.4-1 FLA-2000 and UV Transilluminator Comparison

Left : Image by UV transilluminator (312nm)

Right : FLA-2000

Electrophoresis was carried out on λ DNA/*Hind* III applied to lanes 1-8 in amounts of 100ng, 10ng, 3ng, 1ng, 300pg, 100pg, 30pg and 10pg.

→See Basic SYBR® Green I Data for test protocol.

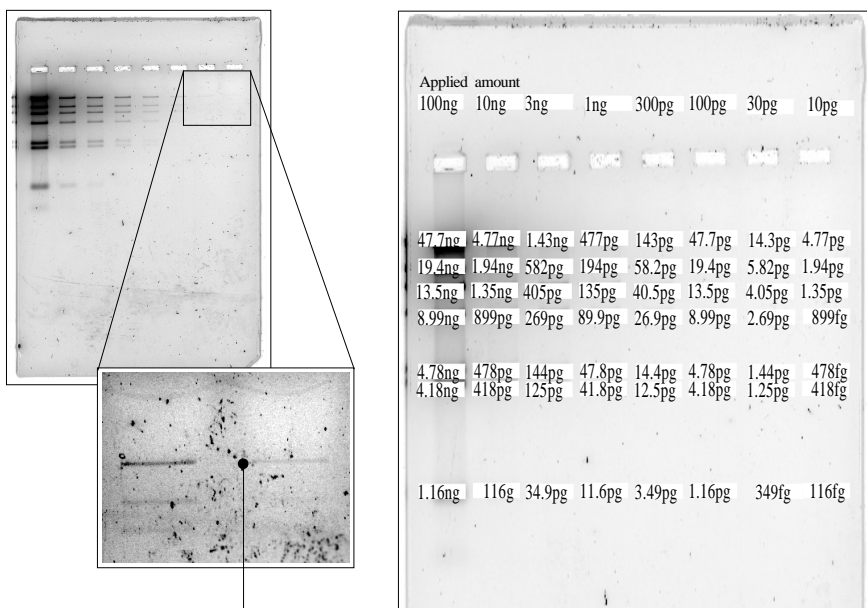
Fig.4-1

The UV transilluminator detected to only 47.7pg while the FLA-2000 detected to 4.77pg, a ten-fold difference in detection sensitivity in favor of the FLA-2000.

■ SYBR® Green I Detection Limit

Detection to 4.77pg was possible using SYBR® Green I with a λ DNA/*Hind* III sample.

SYBR® Green I provides images of lower background than those obtained with EtBr.



4.77pg (zoomed view)

DNA amount of individual bands (zoomed view)

Fig.4-2 FLA-2000 Detection Limit

Same sample as in Fig. 4-1

Fig.4-2

5 One-Point Advice on Analysis Using MacBAS

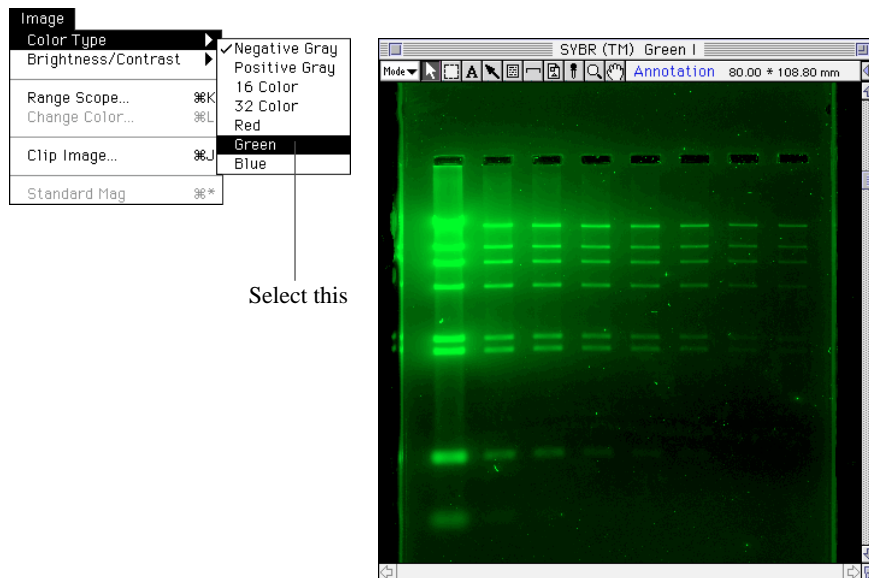
Here is a tip on using the MacBAS analysis software for the FLA-2000 you may find useful.

Q.1 I used the FLA-2000 to read out a sample prepared using SYBR® Green I but would like to get a green image that is more appropriate for SYBR® Green I than the current gray-scale image. Any suggestions?

A.1 With MacBAS it's easy to display data in a color that simulates SYBR® Green I. Just change the color by the following procedure.

Open the SYBR® Green I image, pull down the Image menu, choose Color Type*¹ and then choose Green.

You will get a color display simulating SYBR® Green I.



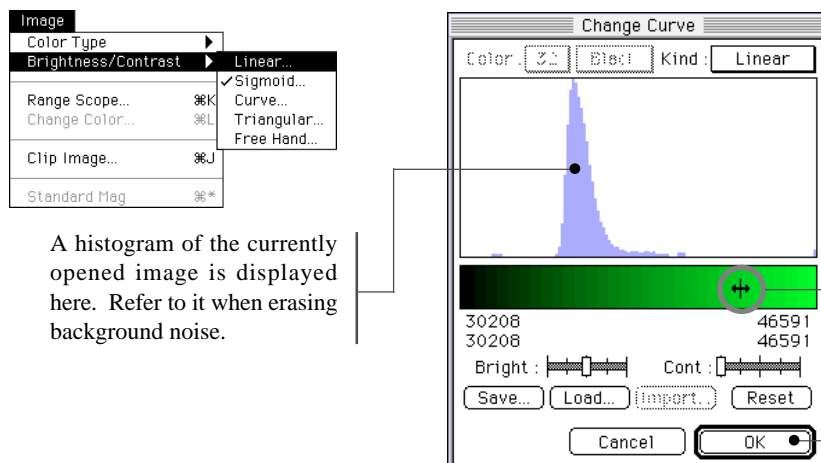
Flow of the analysis

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

To open image
choose File and then Open...

*¹ The initial Color Type setting when the image is opened is Negative Gray.

After choosing Brightness/Contrast*² from the Image menu, choose Linear... You will then be able to get a prettier image by using the Change Curve dialogue to adjust the gradation*³, as explained below.



*² The initial Brightness/Contrast setting when the image is opened is Sigmoid.

*³ This adjustment changes only the displayed image and has no effect on the image data during quantification.

6 References

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2. Piatyszek, M.A., Kim, N.W., Weinrich, S. L., Hiyama, K., Hiyama, E., Wright, W.E., and Shay, J. W. Detection of telomerase activity in human cells and tumors by a telomeric repeat amplification protocol (TRAP), *Methods in Cell Science* 17: 1-15 (1995)
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6. Schmidt, D. M., Ernst J. D. A fluorometric assay for the quantification of RNA in solution with nanogram sensitivity, *Anal. Biochem.* 232: 144-146 (1995)
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