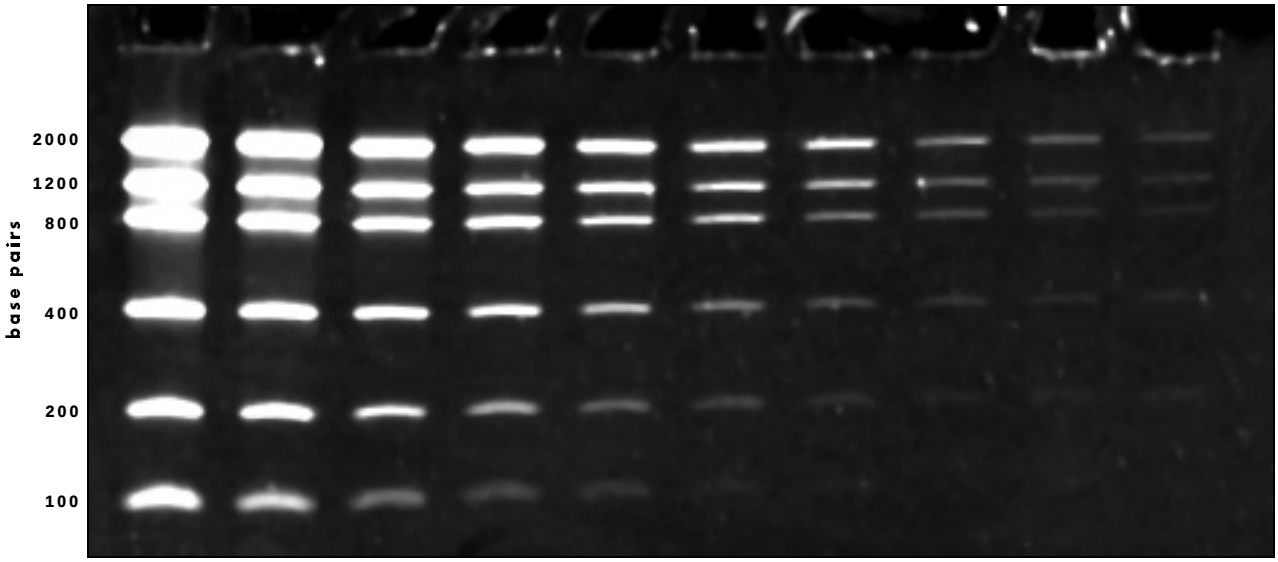
HITACHI
Genetic Systems
Hitachi Software Engineering Co., Ltd.

APPLICATION NOTE

FMBIO® Detection of SYBR® Green I Stained dsDNA in Acrylamide Gels



1 **Two-fold dilution series of Gibco BRL Low Mass Ladder.** 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. Samples were electrophoresed on a precast 10 x 10 x 1 mm 6.0% acrylamide gel (NOVEX) at 100V for 40 minutes, then post-stained in 1:10000 SYBR Green I in 1X TBE, pH 8.0 for 45 minutes. The image was acquired on the FMBIO II Fluorescence Imaging System using a 505/40 nm filter at 100% sensitivity with 256 repeats and a spatial resolution of 300 x 300 dots per inch.

Applications

SYBR® Green I is rapidly replacing ethidium bromide as the fluorescent dye of choice for detecting double-stranded nucleic acids in gels. The FMBIO® II Fluorescence Imaging System can be used to image and quantitate SYBR-stained samples in a wide variety of applications, including:

- dsDNA detection
- Quantitative PCR
- Detection of low copy number DNA and RNA vectors, cosmids, and plasmids
- Detection of phage DNA from cultures with low cell numbers
- Nuclease protection assays
- Bandshift assays

Benefits

SYBR Green I provides the following benefits over EtBr:

- Greater detection sensitivity
- Significantly reduced toxicity[1]
- Lower background fluorescence
- No destaining step needed

The FMBIO II Fluorescence Imaging System provides a number of benefits when imaging SYBR Green I stained gels:

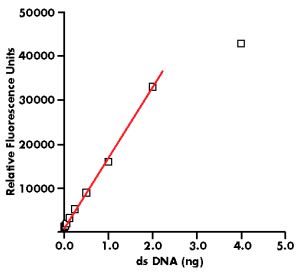
- Fast, easy data acquisition
- 250 times greater detection sensitivity than imaging with a transilluminator and instant film
- Greater dynamic range than instant film or CCD
- Wide variety of quantitation capabilities through FMBIO Analysis Software
- Ability to create, publish, and archive digital images

Sensitivity

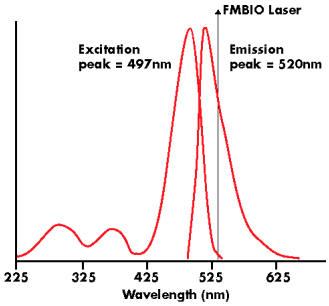
3.9 pg per band

Dynamic Range

2.7 orders of magnitude (3.9 pg/band to 2.0 ng/band)



Dye Spectra



Tips for FMBIO Imaging

1. Many factors contribute to the success of DNA detection[2]. Variables include: gel type and concentration, sample preparation, band size, dye concentration, staining time, gray level settings, and laser focusing point. Further optimization of protocols may result in higher detection sensitivity.

2. For best scanning results, avoid dust specks by using only powderless gloves, rinsing gloves with distilled water, thoroughly cleaning all containers used for staining, and filtering all buffers and solutions with a 0.45µ filter.

3. When scanning, place the gel on a low fluorescence glass plate or sample holder.

4. Loading buffers containing xylene cyanol and bromophenol blue fluoresce strongly when excited by the FMBIO laser, and can interfere with the gel image. For best results, use buffers containing no xylene cyanol, and bromophenol blue concentrations decreased 1:10.

5. Detection sensitivity is higher in acrylamide gels than in agarose gels, due to lower background fluorescence.

6. SYBR Green I is designed for use with double-stranded DNA. For RNA or single-stranded DNA, use SYBR Green II.

7. SYBR stains bind to glass. For storage and staining containers, polypropylene plastics are recommended.

8. Keep solutions containing SYBR Green covered by aluminum foil to prevent bleaching of the dye by ambient light, and store at 4ºC between uses[3]. Stain solution can be reused 3-4 times when stored under these conditions.

9. Although SYBR Green has been found to be significantly less mutagenic than ethidium bromide, it should be handled with the same care given to other nucleic acid stains. See the SYBR Green product insert for instructions on proper handling and disposal.

FMBIO Analysis Software provides easy-to-use features for image analysis:

- Automatic Band Finding
- Band Sizing
- 1D Volume Calculation
- 1D Peak Height
- Lane Traces
- Spot Quantitation
- Background Subtraction
- Standard Curve
- Text and other Annotation
- Gray Level Adjustment
- User-Defined Scan Options

SYBR Green I Ordering Information

Hitachi Genetic Systems, South San Francisco, CA
PBI-11999-29101, 1mL vial
PBI-11999-29150, 500 µL vial

References

1. SYBR® Green I Nucleic Acid Gel Stain. (1996) Molecular Probes Application Sheet MP-7567.

2. Ishino, Y *et al.* (1992) *BioTechniques* **13**, 936.

3. Useful Tips – SYBR® Green I Nucleic Acid Gel Stains. (1997) Molecular Probes Product Information Sheet.

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