



Technical Data

Product evaluation of Midori^{Green} Xtra in DNA staining

Product

Midori^{Green} Xtra (MG10)

Purpose

Evaluate the performance of the new staining reagent Midori Green Xtra by using the in-gel staining method.

Background

One method of staining DNA separated by gel electrophoresis is the “in-gel” staining method. For in-gel staining, electrophoresis is carried out using a gel containing nucleic acid staining reagent. Therefore, it is possible to observe the electrophoresis result without requiring DNA staining process. However, it can come to a distortion of the bands and there is a risk of causing a change in migration pattern, which should be molecular weight dependent. For this reason, in addition of being able to detect the band with high sensitivity, the reagent used for in-gel staining should precisely separate the DNA by size.

<Requirements for in-gel nucleic acid staining reagents>

- High band brightness
- Low background (good S/N [signal to noise] ratio) Advanced detection
- Bands separated according to molecular weight without distortion
- Nucleic acid staining reagents do not affect electrophoretic mobility Accurate size separation

Method

Midori^{Green} Xtra (MGX), which is a new nucleic acid stain reagent, and reagents GG and GR of Company C are used in manufacturer’s specified amount. Gel images were recorded under three light sources of different wavelength (302 nm), Blue (470 nm), and Blue/Green (500 nm). Band formation and band luminance were quantitatively evaluated.

Experimental procedure

① A stained gel was prepared under the following conditions:

- Agarose gel: 2.0% TAE agarose gel (AG02) 12.5 ml / minigel
- Nucleic acid stain reagent: Manufacturer specified amount used

Reagent name	Producer specified amount (2% agarose 100 mL)
Midori ^{Green} Xtra (MG10)	4 μL
Company C stain reagent GG	10 μL
Company C stain reagent GR	10 μL

② DNA dilution series (0.1 μg/μL, 0.05 μg/μL, 0.025 μg/μL, 0.013 μg/μL, 0.006 μg/μL, 0.003 μg/μL) was prepared and 10 μL of each was applied to the prepared gel (right panel).

- DNA sample: 100bp DNA ladder, 0.1 μg/μL (FastGene[®] MWD100)
- Dilution solvent: 10×Loading Dye (TAKARA, 9157) and 1×TAE at a ratio of 1:9.

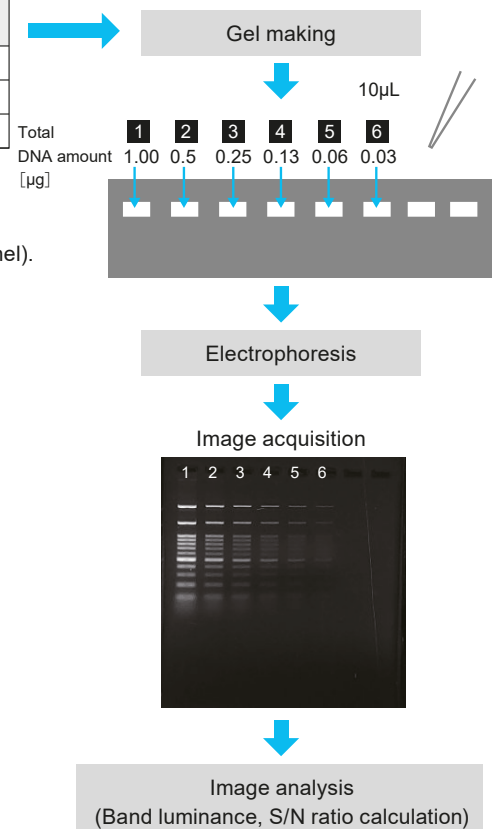
③ Electrophoresis was carried out under the following conditions:

- Electrophoresis: SafeBlue Electrophoresis system (MBE-150Plus)
- Electrophoresis conditions: 100 V, 30 min

④ After Electrophoresis, images of the gel were obtained under following conditions:

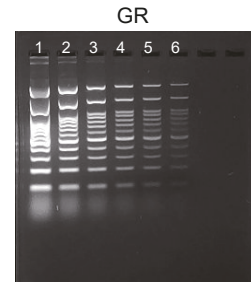
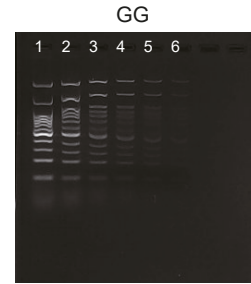
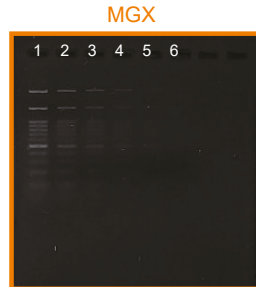
- Capture device: FAS-Digi (Pentax MX-1)
- Shooting conditions: Illuminator
 - I) U.V. (302nm)
 - II) Blue (470nm)
 - III) Blue/Green (500nm)
- Camera : Pentax MX-1
- ISO 100, autofocus, f = 4.0

⑤ Image was analyzed with Image J and the band luminance and S/N ration were calculated for the 100 bp band.

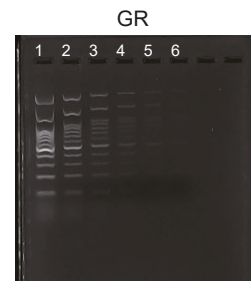
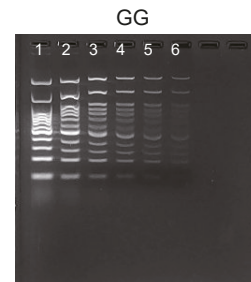


Result

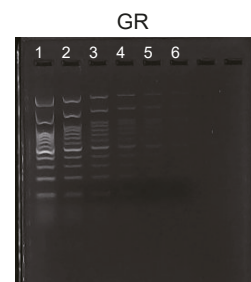
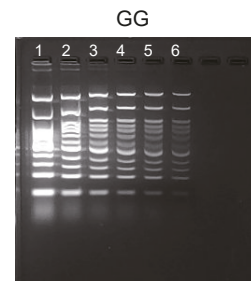
- I) U.V. (302nm)
Exposure time 3 sec



- II) Blue (470nm)
Exposure time 1 sec

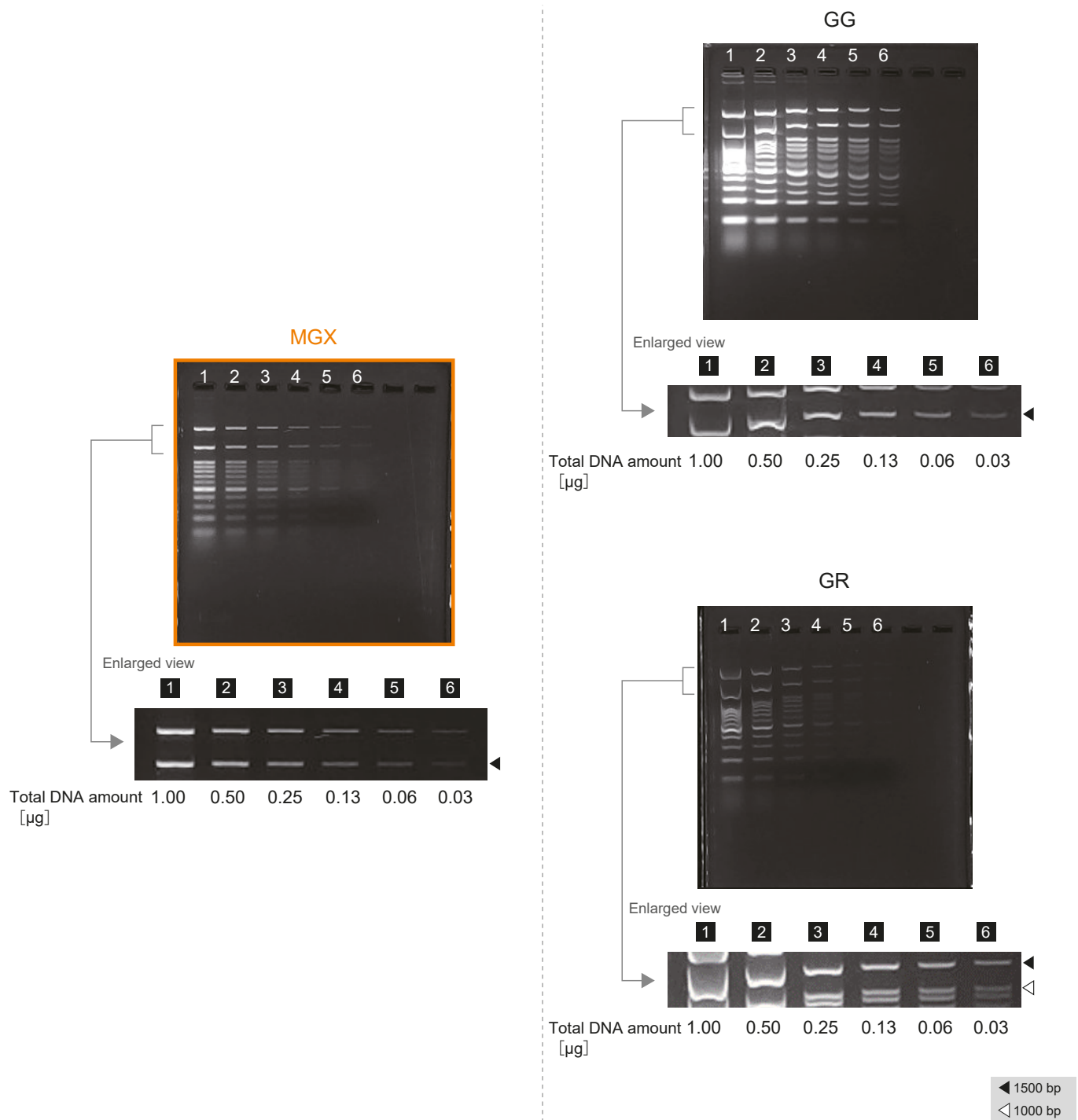


- III) Blue/Green (500nm)
Exposure time 1 sec



1) Influence on band formation

Blue/Green (500nm) Exposure time 1 sec



In MGX

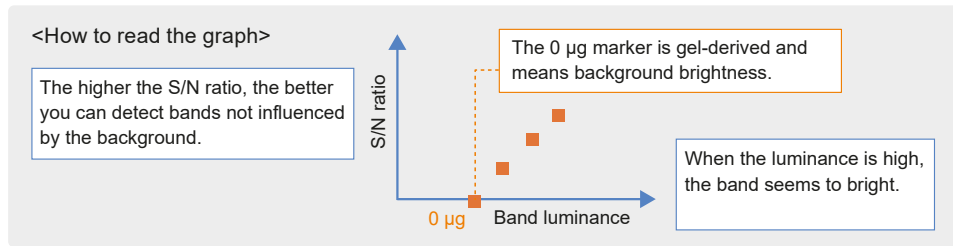
- There is almost no band distortion.

In GG, GR

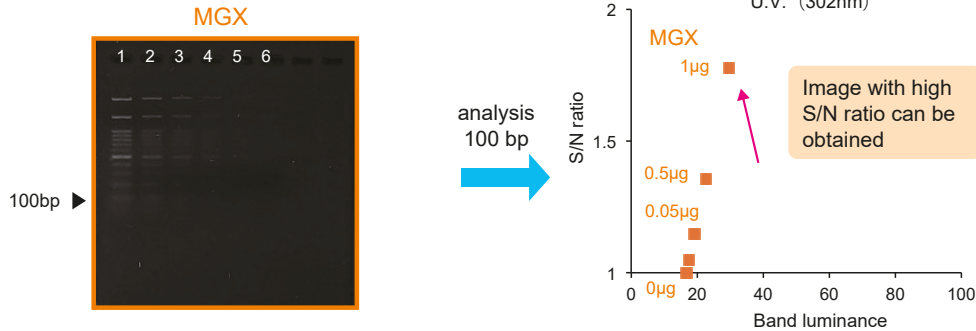
- Changes in electrophoretic mobility depending on DNA amount.
- The band is distorted when the amount of DNA is high.



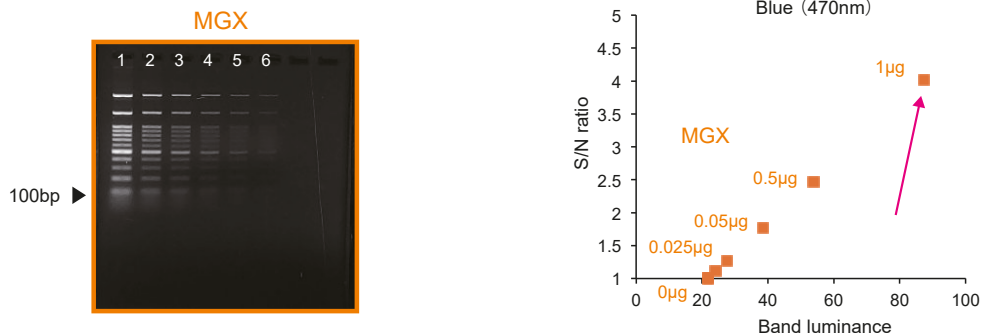
2) About band luminance S/N ratio



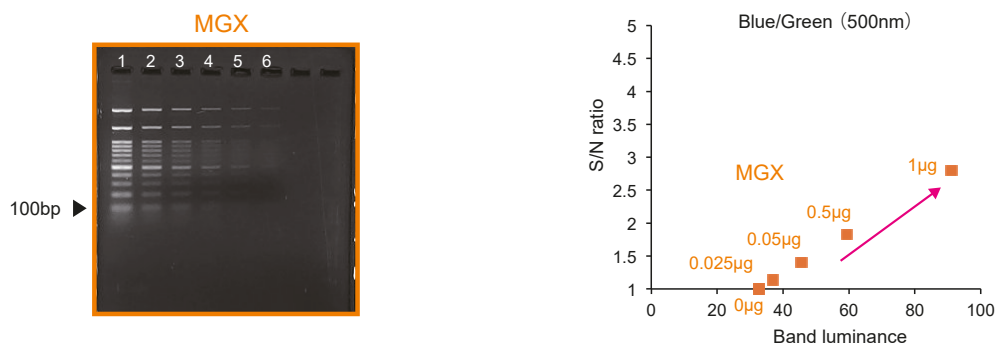
I) U.V. (302nm) Exposure time 3 sec



II) Blue (470nm) Exposure time 1 sec



III) Blue/Green (500nm) Exposure time 1 sec



Summary

- Midori^{Green} Xtra is a reagent with no changes in electrophoretic mobility and band distortion.
- Midori^{Green} Xtra is a DNA staining reagent that enables lower background and higher signal-to-noise ratio.

➔ Midori^{Green} Xtra has the ideal properties for the in-gel staining method with Blue/Green LEDs.