NIPPON Genetics EUROPE

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, elctrophoresis is carried out using a gel containig 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 patern, which should be molecular weight dependent. For this reason, in addition of beeing 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

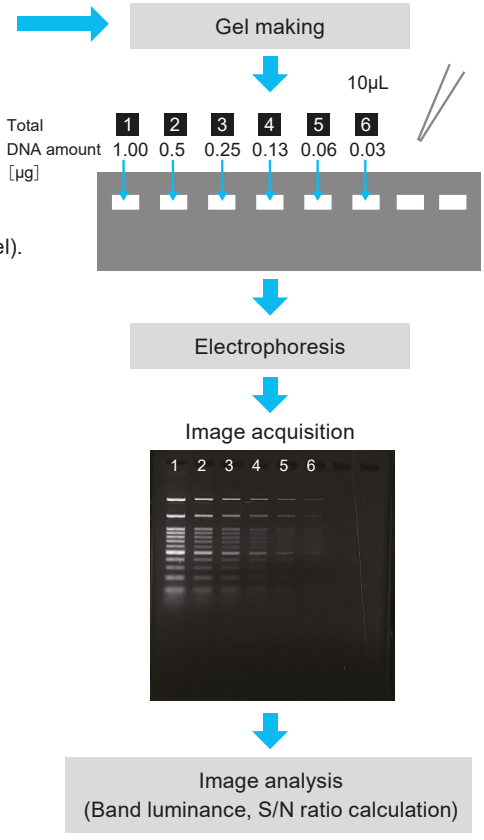
② DNA dilution series (0.1 µg/µL, 0.05 µg/µL, 0.025 µg/µL, 0.013 µg/µL, 0.006 µg/µL, 0.003 µg/µL) was prepared and 10 µL of each was applied to the prepared gel (right panel).
- DNA sample: 100bp DNA ladder, 0.1 µg/µL (FastGene® MWD100)
- Dilution solvent: 10×Loading Dye (TAKARA, 9157) and 1×TAE at a 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.

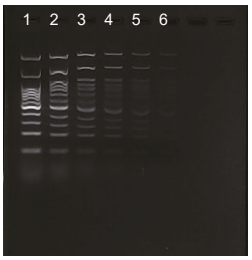


Result

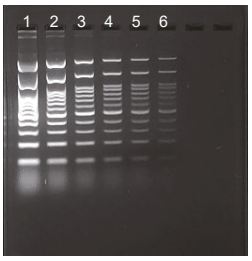
I) U.V. (302nm)
Exposure time 3 sec

MGX

GG



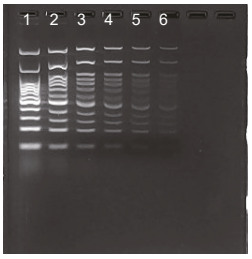
GR



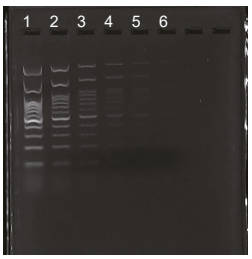
II) Blue (470nm)
Exposure time 1 sec

MGX

GG

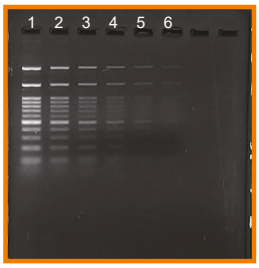


GR

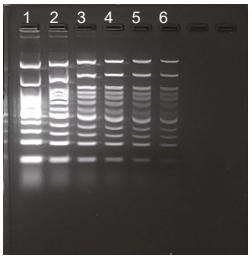


III) Blue/Green (500nm)
Exposure time 1 sec

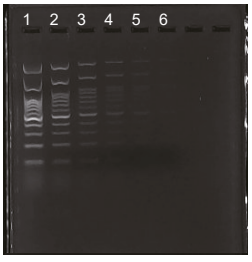
MGX



GG



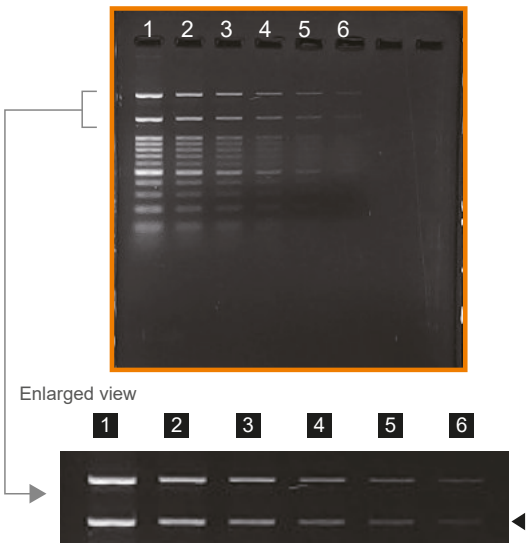
GR



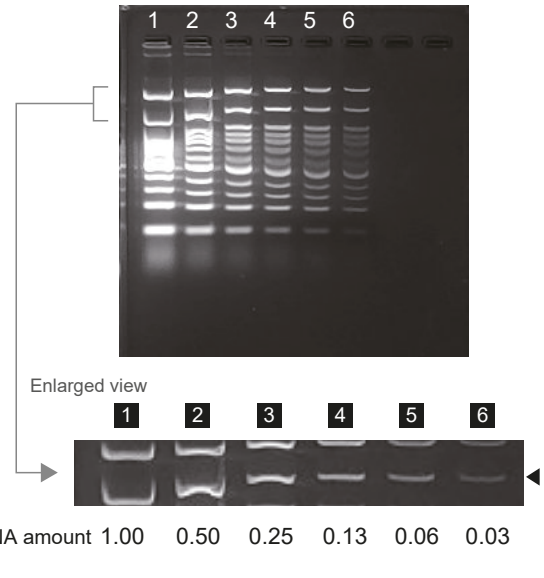
1) Influence on band formation

Blue/Green（500nm） Exposure time 1 sec

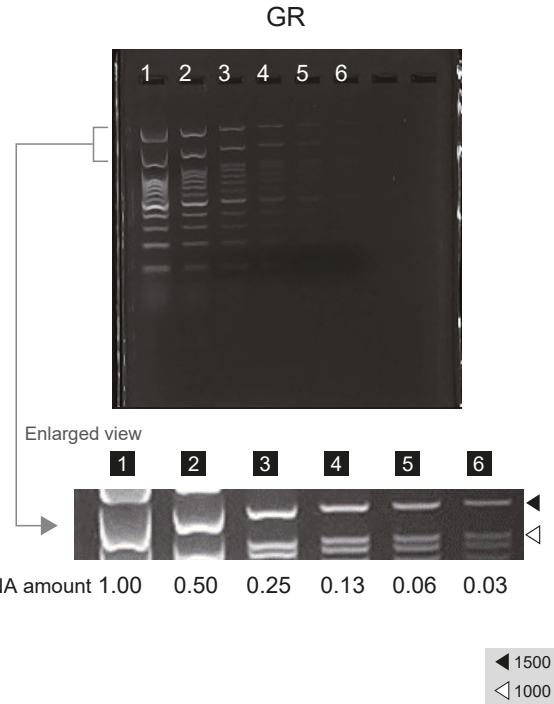
MGX



GG



GR

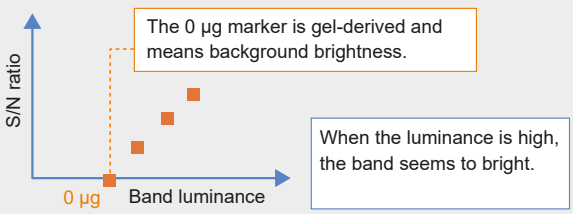


In MGX	In GG, GR
• There is almost no band distortion.	• Changes in electrophoretic mobility depending on DNA amount. • The band is distorted when the amount of DNA is high.

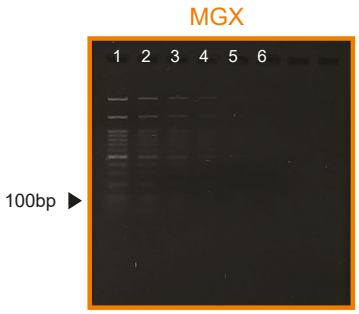
2) About band luminance S/N ratio

<How to read the graph>

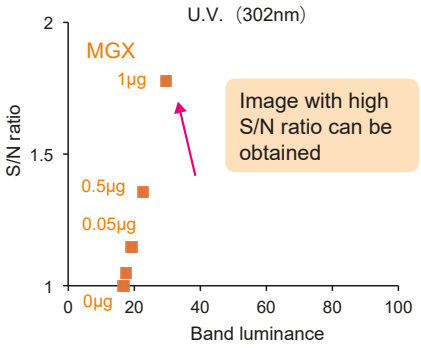
The higher the S/N ratio, the better you can detect bands not influenced by the background.



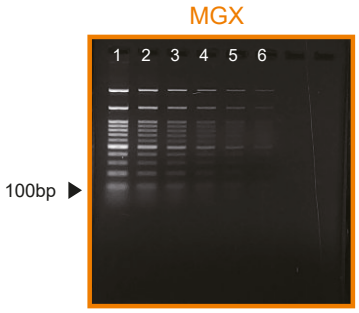
I) U.V. (302nm) Exposure time 3 sec

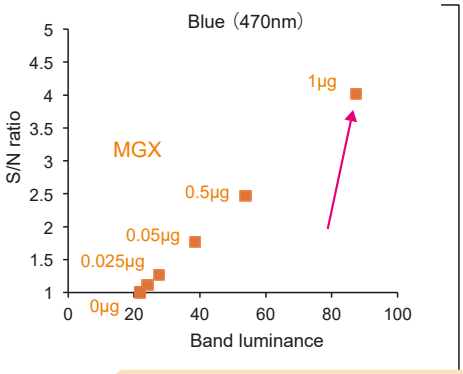


analysis 100 bp



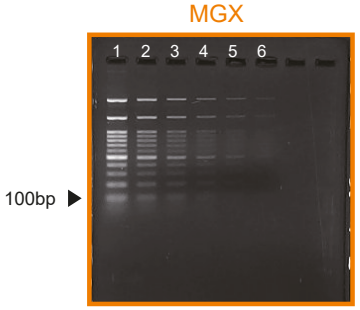
II) Blue (470nm) Exposure time 1 sec

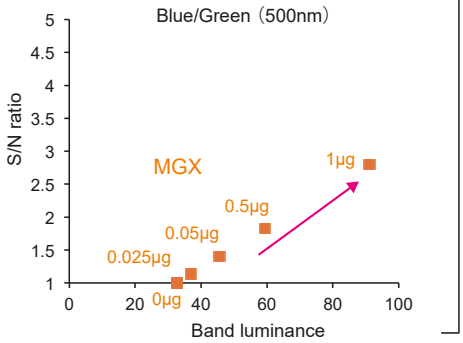




Low Background
Both S/N ratio and brightness improved

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.

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