



Technical Data

Product Evaluation of Midori Green Xtra post-staining method

Product

FastGene® Midori Green Xtra (Cat.No. MG10)

Purpose

Midori Green Xtra (MGX) is an excellent staining reagent for DNA pre-staining. Comparative test with other DNA staining reagents were carried out to evaluate the performance in DNA post-staining method.

- Staining time dependent degree of DNA staining (→ result 1)
- Difference in appearance of DNA bands according to excitation wavelength (→ result 2)



Midori Green Xtra

DNA staining reagent with properties ideal for DNA pre-staining.

➔ There is no distortion of the bands. There is no shift in mobility depending on the amount of DNA.

Best reagent for observation under Blue/Green LED, Blue LED illumination

➔ Low background, high S/N ratio band image

For details on product evaluation using DNA pre-staining, please see Technical Note 2018_06

Experimental procedure

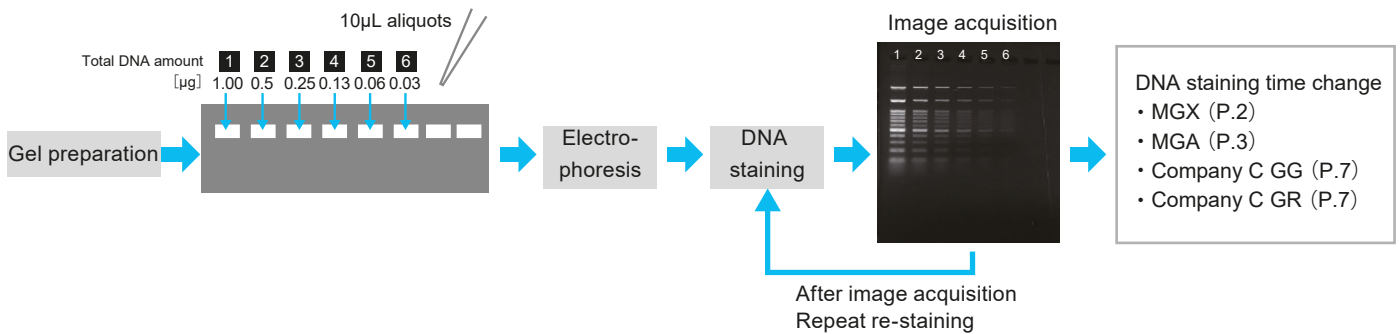
- ① A 2% agarose gel (12,5 mL /mini gel) was prepared.
 - Reagents: Agarose (FastGene®, AG02)
 - Solvent: 1 x TAE buffer
- ② Dilution series of DNA (0.100, 0.050, 0.025, 0.013, 0.006 µg/µL) were prepared and 10 µL each was applied to the gel.
 - DNA sample: 100bp DNA ladder (0.100 µg/µL, FastGene®, MWD100)
 - Dilution solvent: A mixture of 10x Loading Dye (TAKARA, 9157) and 1x TAE buffer in a ratio of 1:9
- ③ Electrophoresis was performed independently for evaluation of each staining reagent.
 - Electrophoresis device: SafeBlue Electrophoresis system (Major Science, MBE-150Plus)
 - Electrophoresis conditions: 100 V, 35 min
- ④ DNA post-staining was performed under the following conditions.

Staining reagent	Amount used in 100 mL TAE	Recommended amount
Midori Green Xtra(MGX), FastGene®, MG10	10 µL	10 µL
Midori Green Advance (MGA), FastGene®, MG04	10 µL	10-25 µL
Company C staining reagent GG	30 µL	30 µL
Company C staining reagent GR	30 µL	30 µL

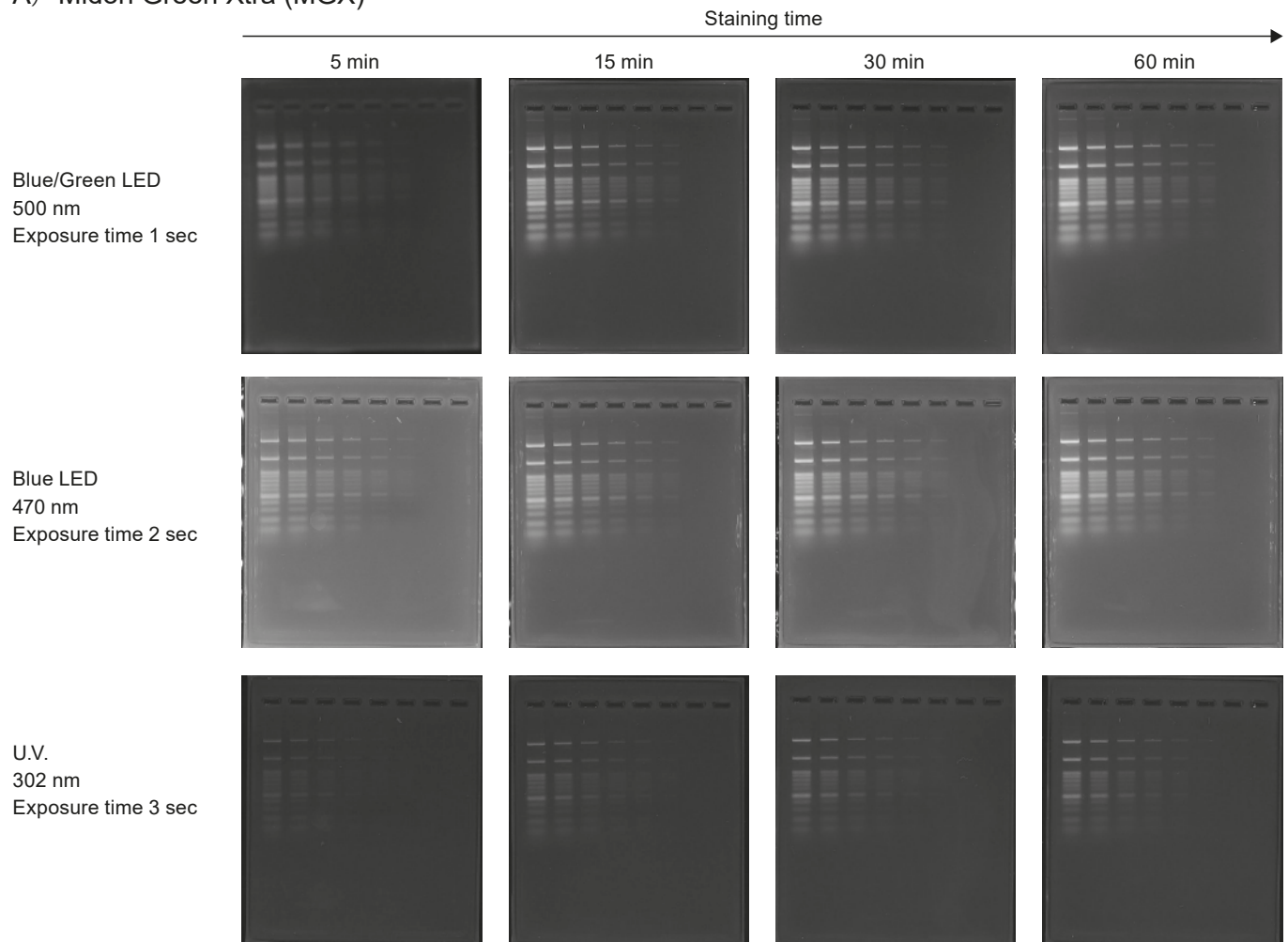
- ⑤ After electrophoresis, an image of the gel was obtained under the following conditions:
 - Detection device: FAS-Digi (Pentax MX-1)
 - Detection conditions: Illuminator
 - i) Blue/Green LED 500 nm
 - ii) Blue LED 470 nm
 - iii) U.V. 302 nm
 - Camera: Pentax MX-1
 - ISO 100, autofocus, f =4.0

Result 1: Change in DNA staining time

The appearance of DNA bands at DNA staining times after 5, 15, 30, 60 min was compared.



A) Midori Green Xtra (MGX)



Result

<Result A1> An increase in band brightness was observed from 5 minutes till 15 minutes of staining time.

<Result A2> The band brightness was slightly increased from 15 minutes to 30 minutes.

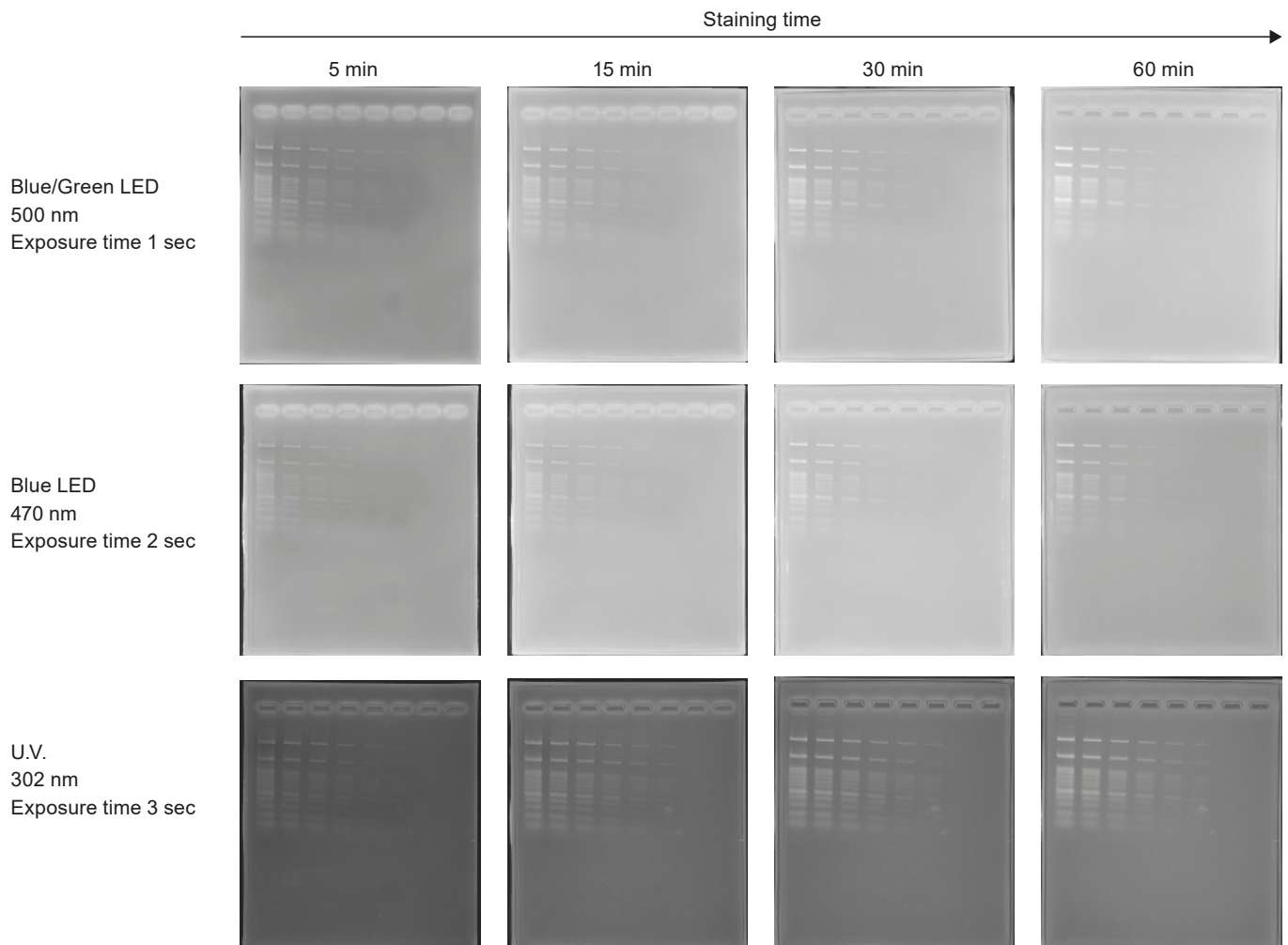
<Result A3> Almost no change in band brightness was observed from 30 minutes to 60 minutes of staining time.

<Result A4> No significant increase in background was consistently observed between 5 min and 60 min of staining time.

Conclusion

- If DNA post-staining is performed at an MGX concentration of 10 µL/ 100 mL (recommended condition by manufacturer), DNA staining with MGX is completed by 30 minutes of staining time <Results A1, A2, A3>.
- MGX has high specificity to DNA and high contrast image can be obtained <Result A4>.

B) Midori Green Advance (MGA)



Results

<Result B1> An increase in band brightness was observed from 5 minutes to 15 minutes of staining time.

<Result B2> The band brightness was slightly increased from 15 minutes to 30 minutes.

<Result B3> Almost no change in band brightness was observed from 30 minutes to 60 minutes of staining time.

<Result B4> The influence of the background is strong, and the tendency becomes stronger as the staining time is longer.

Conclusion

- When DNA post-staining is performed at an MGA concentration of 10 μ L/ 100 mL (recommended condition by manufacturer), DNA staining by MGA is completed by 30 minutes of staining time <Results B1, B2, B3>.
- The MGA has a risk that background effects may appear strongly depending on the conditions of image acquisition <Result B4>.

C) Based on competitor product comparison

Binding to DNA was almost completed by the staining time of 30 min even with other staining reagents GG and GR (see "Additional information: Changes in DNA staining time of the competitor's nucleic acid staining reagent" see this document p.6)

Therefore, the staining completion time of MGX and MGA was almost the same as GG and GR.

Conclusion

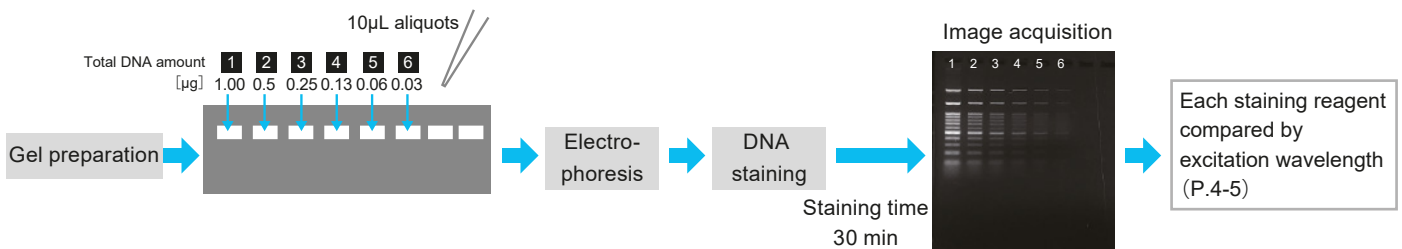
The recommended amount of MGX for DNA post-staining is 10 μL / 100 mL, which is smaller than the competitor amount (30 μL / 100 mL).

However, there is no significant difference in the time required for staining in any of the nucleic acid staining reagents, and the binding to DNA is almost completed by 30 minutes of staining time.

Staining reagent	Amount in 100 mL TAE	Main staining time	Estimated staining time for completion
Midori Green Xtra	10 μL	5, 15, 30, 60 min	30 min
Midori Green Advance	10 μL	5, 15, 30, 60 min	30 min
Competitor C GG	30 μL	5, 15, 30, 60 min	30 min
Competitor C GR	30 μL	5, 15, 30, 60 min	30 min

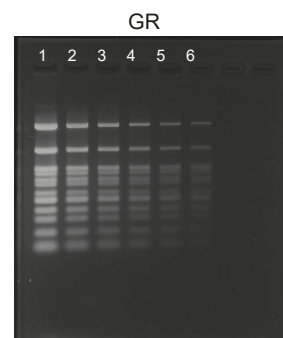
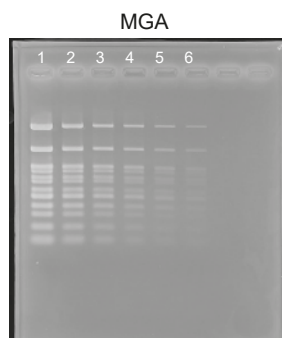
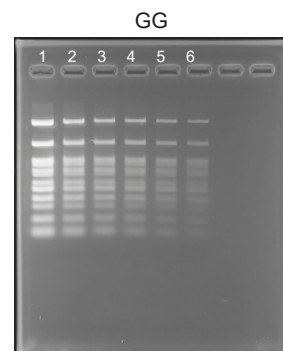
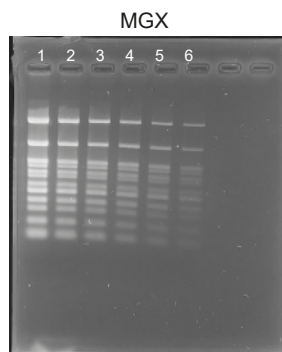
Result 2: Comparison of each staining reagent by excitation wavelength

The appearance of DNA bands stained with each nucleic acid staining reagent at a DNA staining time of 30 minutes was compared.



A) Blue/Green LED (500 nm) Exposure 1 sec, ISO 100

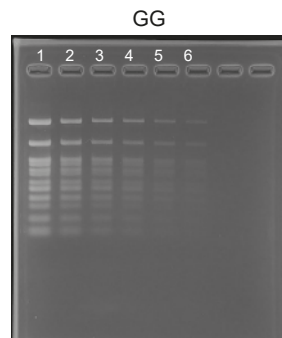
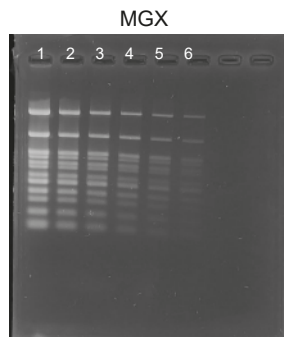
MGX has a low background, and bright bands



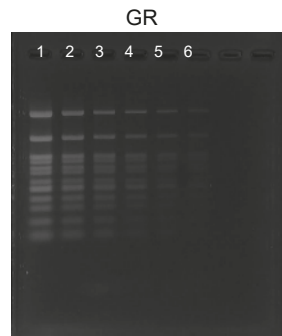
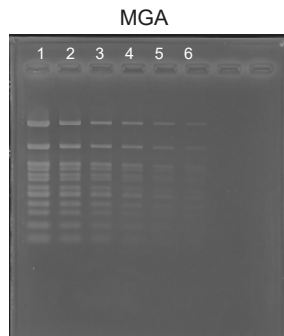
The MGX band is bright even when compared to other stains

B) Blue LED (470 nm) Exposure 2 sec, ISO 100

MGX has low background and bright bands

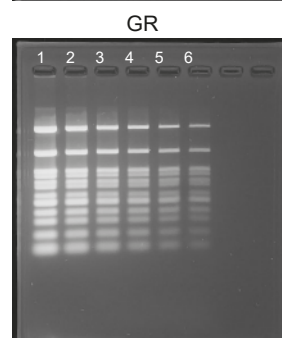
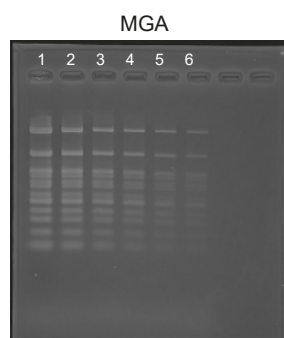
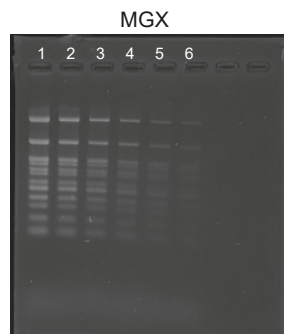


The MGX band is bright even when compared to other stains



C) U.V. (302 nm) Exposure 3 sec, ISO 100

MGX has dark bands at UV



Conclusion

MGX produces sharp DNA band images with Blue LED (470 nm) as well as Blue/Green LED (500 nm).

Recommended/Compatible Table for Illuminator (Light Source Wavelength) when using Post-Staining Method.

	Post-Staining Method		
	Blue/Green LED (500 nm)	Blue LED (470 nm)	U.V. (302 nm)
Midori Green Xtra	◎	◎	△ (Dark)
Midori Green Advance	○ (High Background)	○	○
Company C GG	◎	◎	○
Company C GR	◎	△ (Dark)	◎

Summary



Midori Green Xtra



DNA staining reagent with properties ideal for DNA pre-staining.

➔ There is no distortion of the bands. There is no shift in mobility depending on the amount of DNA.

Best reagent for observation under Blue/Green LED, Blue LED illumination

➔ Low background, high S/N ratio band image

For details on product evaluation using DNA pre-staining, please see technical note 2018_06

High performance in DNA pre-staining

Recommended/compatible Illuminator when using Pre-staining Method

	Staining Method		
	Blue/Green LED (500 nm)	Blue LED (470 nm)	U.V. (302 nm)
Midori Green Xtra	◎	◎	△ (Dark)
Midori Green Advance	○	○	○
Company C GG	× (Band distortion)		
Company C GR	× (Band distortion)		

High performance in DNA post-staining method

Result in this document

1. Time for completion of staining

Staining reagent	Amount used in 100 mL TAE (recommended)	Recommended time	Estimated time from this experiment
Midori Green Xtra	10 µL (10 µL)	10-30 min	30 min
Midori Green Advance	10 µL (10-25 µL)	5-60 min	30 min
Company C GG	30 µL (30 µL)	-30 min	30 min
Company C GR	30 µL (30 µL)	-30 min	30 min

2. Recommended/compatible Illuminator when using DNA post-staining method

	Post-staining method		
	Blue/Green LED (500 nm)	Blue LED (470 nm)	U.V. (302 nm)
Midori Green Xtra	◎	◎	△ (Dark)
Midori Green Advance	○ (High background)	○	○
Company C GG	◎	◎	○
Company C GR	◎	△ (Dark)	◎

Supplemental information: DNA staining time change of competitor's nucleic acid stain

