

Product: **MIDORI Green Advance (MG04)**
 Manufacturer: **NIPPON Genetics Co., Ltd**
 Application: **Detection of pathogenic ribosomal RNA in fish tissue and in the environment**

The here presented data was provided by the courtesy of Dr. Zenke Kosuke, Laboratory of Fish Diseases, Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan

Introduction

Our laboratory, specialized the diagnosis of fish disease, uses PCR to detect the presence of disease specific genes. The results are analyzed using gel electrophoresis. Therefore an accurate DNA migration is crucial. Additionally, the combination of DNA stains with different light sources can generate a background signal which reduces the sensitivity. There, the DNA stain Midori Green Advance was investigated as an alternative for Ethidium Bromide. Furthermore, the ability to use ultraviolet radiation and the adjustment of the gel's thickness to decrease the background were analyzed.

Methods

● Electrophoresis conditions

| | |
|-------------------------|---|
| Instrument: | Mupid exU |
| Gel: | 1.0% FastGene Agarose (AG01) in 0.5 x TAE |
| Electrophoresis buffer: | 0.5xTAE |
| Voltage: | 100V |
| Time: | 30min |
| Gel Thickness: | 0.5mm |
| Gel size: | 60mm x 110mm |
| Volume: | 30mL |

● Sample

| | |
|--------------|--------------------------------------|
| PCR Product: | 1.9kb (Potential infection) 2.0kb |
|--------------|--------------------------------------|

● Staining

Precast: 5 µl of MIDORI Green Advance were diluted in 100 ml 0.5 x TAE Buffer

Post-staining: 10 µl MIDORI Green Advance were diluted in 100 ml 0.5 x TAE buffer. Gel was stained for 20 min, shaking.

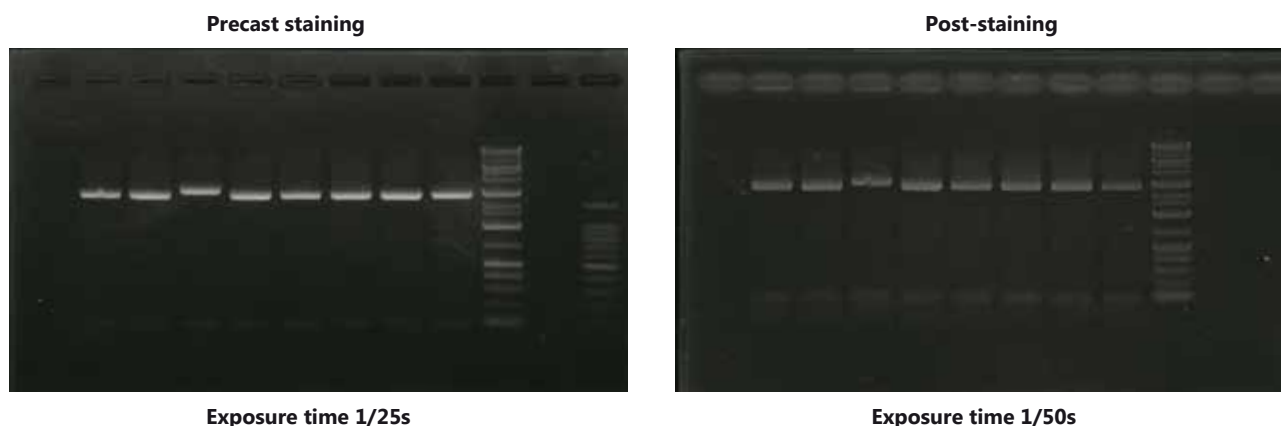
No destaining was necessary

● Image recording

| | |
|---------------------------|---------------------------|
| Illuminator | |
| UV transilluminator | 302nm |
| Blue LED illuminator | 470nm (FG-05) |
| Imaging Instrument | FAS-Digi (GP05LED) |
| Settings | Aperture F2.5 ISO 3200 |
| Exposure time | 1/10, 1/25, 1/50s |

Results


Analyzing DNA mobility using MIDORI Green Advance in prestained gels or after post-staining.



● Summary

These results suggest using MGA and UV did not affect the migration and got good sensitivity.

Comment from NIPPON Genetics: Post-staining in electrophoresis in no risk of problem related to the migration. In this experiment, precast staining showed the same pattern as post-staining.

Details on next page 

Observing the the gel under various conditions when using MIDORI green Advance

UV (302nm)

| | | | |
|------------------|--------|--------|--------|
| Precast staining | | | |
| Post staining | | | |
| Exposure time | 1/50 s | 1/25 s | 1/10 s |

Blue LED (470nm)

| | | | |
|------------------|--------|--------|--------|
| Precast staining | | | |
| Post staining | | | |
| Exposure time | 1/50 s | 1/25 s | 1/10 s |

●Summary

MIDORI Green Advance showed an almost backgroundless image and accurate migration when using UV-light. The background with blue LED light was slittly increased. Therefore, the sensitivity was a decreased but it was still usable.

Comment from NIPPON Genetics:

Ultraviolet light is shortwave/energy rich light source which is known to damage the DNA. Please also see our technical note "Blue/Green LEDs - Sensitivity with different DNA staining dyes" showing superior signal intensity with safe lights.

<Customer's comment>

In the past, we have used DNA dyes which were directly mixed with the sample. There was however one case in which the DNA band migration pattern was changed. This problem did not occur when using Ethidium Bromide. However, EtBr has the safety issue so we tried other gel dyeing reagents. These delivered poor sensitivity.

Using MIDORI Green Advance, both issues, migration shift and sensitivity, could be addressed. In addition pre- and poststaining can be performed enabling a shorter experimental time.