NIPPON Genetics EUROPE

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

●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

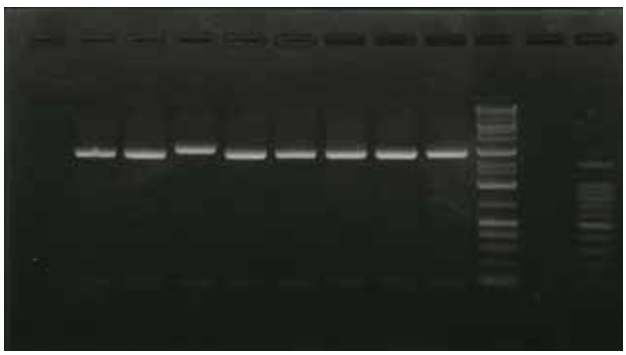
●Staining

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

Results

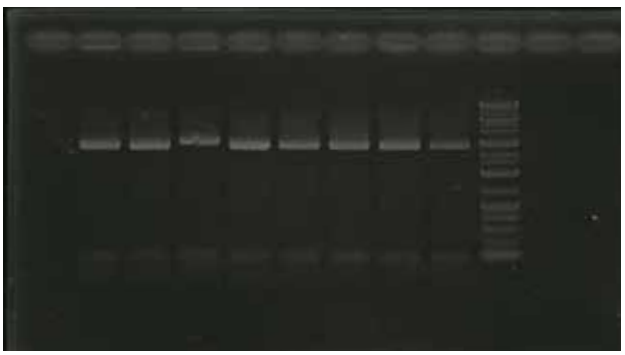
Analysing DNA mobility using MIDORI Green Advance in prestained gels or after post-staining.

Precast staining



Exposure time 1/25s

Post-staining



Exposure time 1/50s

●Summary

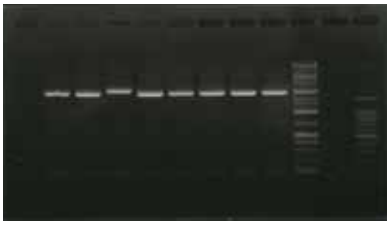
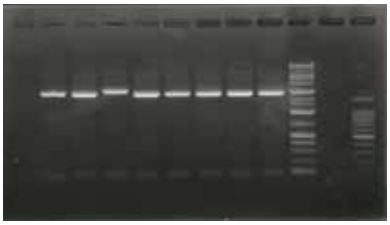
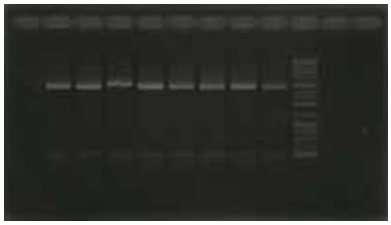
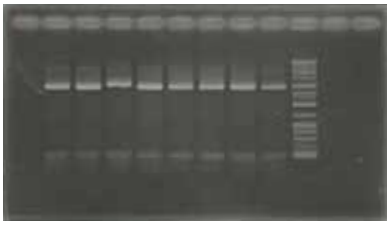
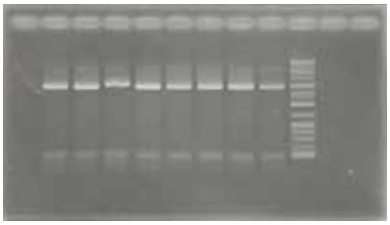
These results suggest using MGA and UV did not affect the migration and got good sensitivity.

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.

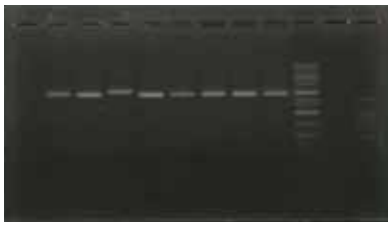
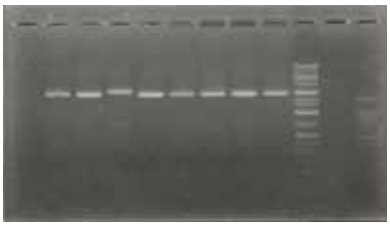
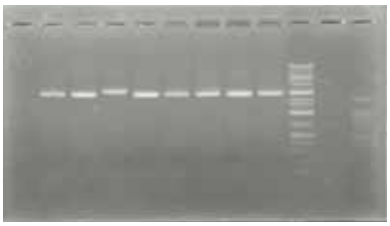
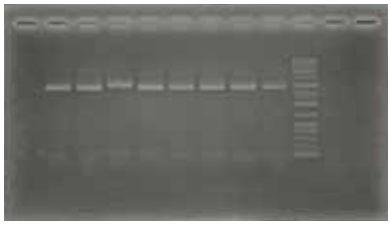
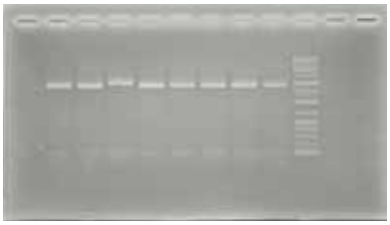
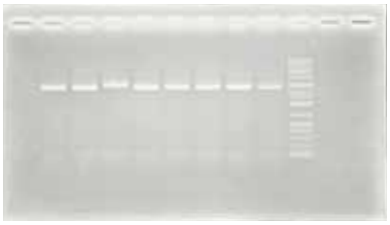
Details on next page

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.