NIPPON Genetics EUROPE

Application

Cleavage of plasmid with restriction enzyme and confirmation of DNA fragments

Product: Midori Green Advance (Cat No. MG04)
Manufacturer: NIPPON Genetics EUROPE

Product: FastGene® FAS-Digi (Cat No. GP-05LED)
Manufacturer: NIPPON Genetics EUROPE

The following data was published due to the kindness of Mr Naoharu Takano and Mr. Shota Moriya, Biochemistry Dept., Tokyo Medical University, Japan.

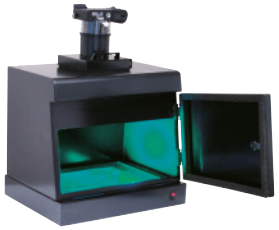
Purpose

In student training, this experiment was carried out in order to teach how to draw maps of plasmids and the function of restriction enzymes. A plasmid was cleaved by restriction enzyme treatment and the length of the DNA fragments was confirmed by electrophoresis.

Plasmid (sample)
↓
Restriction enzyme treatment
↓
Electrophoresis
↓
Gel photography

Nucleic acid stain
Midori Green Advance
(Cat No. MG04)

Gel Documentation System
FastGene® FAS-Digi
(Cat No. GP-05LED)

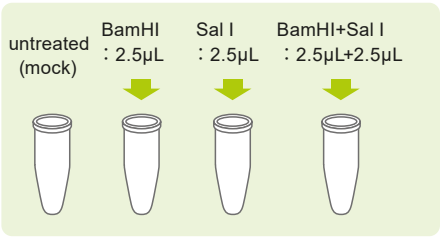


Experimental Method

Prepare the following solution in a 1.5 mL tube.

Plasmid (sample) (100 ng/μL)	5 μL
TOYOBO 10×H buffer	5 μL
Ultrapure water (H2O)	40 μL
total	50 μL×4

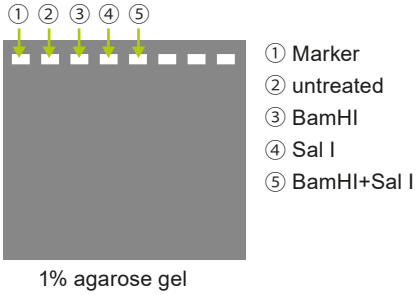
Plasmids are treated with four enzymes.



SalI, BamHI: TOYOBO

2.5 μL of restriction enzyme is added to the tube prepared above.
↓
Tap and mix well.
↓
Centrifuge with mini centrifuge: 5 sec
↓
Incubation at 37°C. Water bath for 1 h.
↓
Add 5 μL of 10x loading buffer (Nippon Gene: 313-90111)
↓
Tap and mix well.
↓
Apply the sample to the dyed gel as shown on the right.
↓
Electrophoresis: 100 V
Buffer: TBE
↓
When the blue pigment, contained in the loading buffer, reaches 1/2 of the whole gel, the electrophoresis is terminated. (Observe DNA band with LED transilluminator.)
↓
Perform gel imaging and check results.

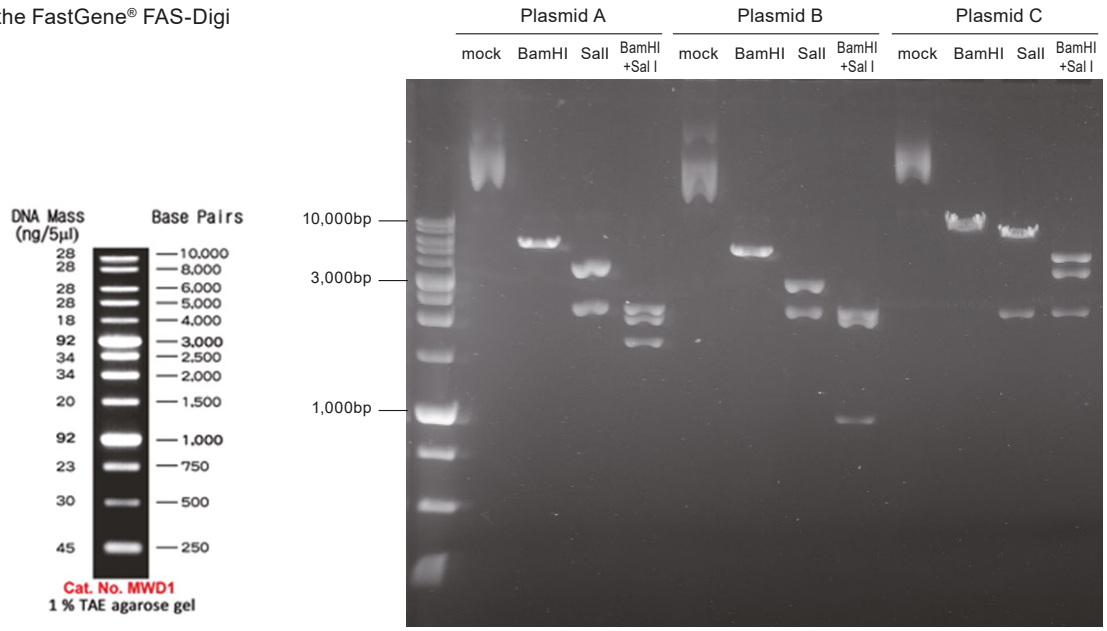
Mini centrifuge
NG002B



1% agarose gel

Results

Shot with the FastGene® FAS-Digi



Customer`s comment

I used it for student training, to teach how to draw plasmid maps and the function of restriction enzymes. With EtBr, there was a problem, that the contamination area of EtBr expands during gel staining, but there is not such a problem while using Midori Green, because it is safe and results can be detected immediately. I was able to save time for cleaning up after styining by using a safe dye.

It is not bad that it is possible to stop the electrophoresis at the right place, because we can observe the gel live with LEDs while doing the electrophoresis, although staining of the gel is weakened for low molecular side, when the gel was pre-stained.

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