Application

Remove DNA of plant sample-derived cloning inhibitors from agarose gel

Product FastGene® Gel / PCR Extraction Kit（FG-91202, FG-91302）

Manufacturer NIPPON Genetics EUROPE

The following data was kindly provided by Mr. Kohei Ogura, Business Development Department (Seeds and Seeds Inspection), Fasmac Co., Ltd., Japan

Method

● Purpose

In order to remove low molecular regions that would inhibit cloning, the gel was cut out and purified. Only the DNA of the targeted higher molecular regions was collected.

The purified sample was cloned and a library was prepared. Sequencing and homology analysis were performed afterwards.

● Sample condition

Type ：Plant leaves 2 types

Treatment ：After nucleic acid extraction, RT-PCR, total nucleic acid amplification, gel extraction

Amount used ：about 200 mg of agarose gel

● Amount of elution buffer used:

50µL

● Procedure

1. RT-PCR was performed after nucleic acid extraction
2. Electrophoresed with 2% TAE agarose, 100 V for 20 min to separate DNA fragments
3. The target fragment was cut out and transferred to a 1.5 mL Eppendorf tube
4. DNA was recovered using FastGene® Gel/PCR Extraction (FG-91302)
5. Measured concentration of DNA solution after elution with 50 µL of GP3 buffer
6. The collected DNA sample was electrophoresed

Result

● Electrophoretic results of the DNA before and after purification. The results of concentration and purity are as followed:

DNA concentration / purity（Sample A ／ B）

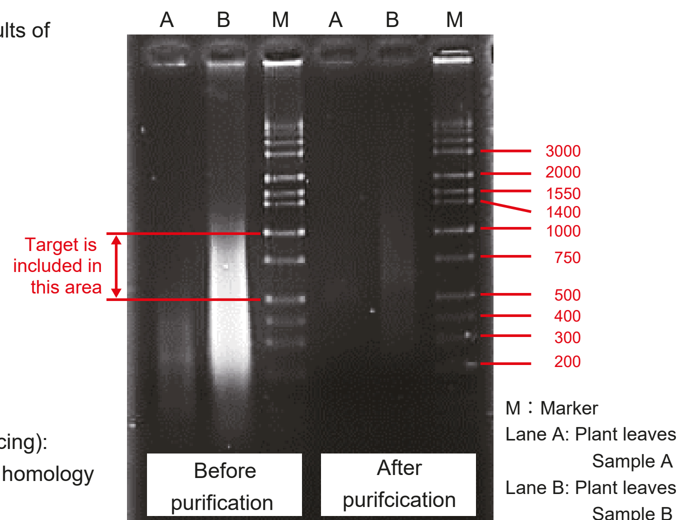
	Before（100 µL Buffer）		After（50 µL GP3）	
	Sample A	Sample B	Sample A	Sample B
Conc.（ng/µl）	1186.7	1037.1	0.01	13.17
260/280 nm	1.80	1.76	0.00	1.97
260/230 nm	1.93	1.84	0.00	15.24

Gel electrophoresis condition:

10 µL per lane, TAE buffer, 2% agarose, 100 V for 20 minutes

Data and test conditions for downstream applications (such as sequencing):

Cloning with the purified sample, create library, sequence and perform homology analysis.



※ Use 10 µL of the sample eluted with 50 µL for electrophoresis.

Customer's comment

This time, this kit was used to excise and purify the gel in order to obtain only the region (500-1000 bp) containing the target after total nucleic acid amplification. As a matter of course, this step increases the cloning efficency (establishment of target insertion), and also increases the detection rate of the target sequence data in homology analysis.

Sample A shows almost zero recovery after purification, as the target region was hardly amplified. On the other hand, sample B has the opposite result to sample A, because the target area was amplified.

Copyright© NIPPON Genetics Europe, All Rights Reserved. 2016.JAN

Binsfelder Straße 77,
52351 Düren, Germany

www.nippongenetics.eu

TEL +49 2421 554960 FAX +49 2421 55496 11 info@nippongenetics.eu