NIPPON Genetics EUROPE

Application

Cloning of isoflavone biosynthesis related enzyme using RNA extracted from soybean

Product: FastGene® Scriptase Ⅱ cDNA Synthesis Kit（LS63）

Manufacturer: NIPPON Genetics EUROPE

The data was kindly provided by Mr Ryo Mameda at Mr. Nakayama Laboratory, Department of Biomolecular, Graduate School of Engineering, Tohoku University, Japan

Overview

Plants adapt to changes in the enviroment by biosynthesizing specialized metabolites (secondary metabolites). In order to study the physiological functions of these substances, investigation of metabolic enzymes that biosynthesize them is essential. Here, we focused on soy isoflavone and conducted an experiment aimed at gene cloning of an isoflavone biosynthesis related enzyme.

Experimental conditions

- Initial sample：Soybean (Glycine max) suspension culture cells 100 mg
- RNA purification：RNAiso plus (TaKaRa)

Flow

Reverse Transcription

Reverse transcriptase：
FastGene® Scriptase II
cDNA Synthesis Kit

■ Advantages
- Obtain longer cDNA due to lower RNase H activity
- Multifunctional Enzyme optimized for qPCR and NGS

PCR reaction

PCR enzyme：
KOD FX Neo (TOYOBO)
PCR Cycler：
PCR Thermal Cycler Dice (TaKaRa)

Cloning

Electrophoresis

Gel imaging device：
TF-20L (Vilber Lourmat)
Nucleic acid staining reagent：
Ethidium bromide

Sequencing

Protocol

1) 1 µL of oligo dT primer and 1 µg of total template RNA were mixed
2) 2 µL of dNTP mixture (2 mM each) was added
3) distilled Water (DW) was added to a total volume of 12.5 µL
4) Incubation at 65°C for 5 minutes, afterwards cool on ice immediately (denaturation)
5) The following components were added

5x FastGene® Scriptase II buffer	4	µL
0.1 M DTT	2	µL
RNase Inhibitor	0.5	µL

6) Incubation at 42°C for 2 minutes (primer annealing)
7) 1 µL of FastGene® Scriptase II was added to RNA mixture on ice
8) Incubation at 42°C for 50 minutes (activation of reverse transcriptase)
9) Incubation at 70°C for 15 minutes (inactivation of reverse transcriptase)

10) PCR amplification of synthesized cDNA

● Reaction composition

DW	10	µL
2x PCR Buffer for KOD FX Neo	25	µL
2 mM dNTPs	10	µL
Primer (10 µM each)	1.5	µL
Template (synthesized cDNA)	1	µL
KOD FX Neo (1 U/µL)	1	µL
Total	50	µL

● PCR program

94℃	2 min	(Initial denaturation)
↓		
98℃	10 sec	(Denaturation)
↓		
55℃	30 sec	(Annealing)
↓		
68℃	1 min	(Elongation)

30 cycles

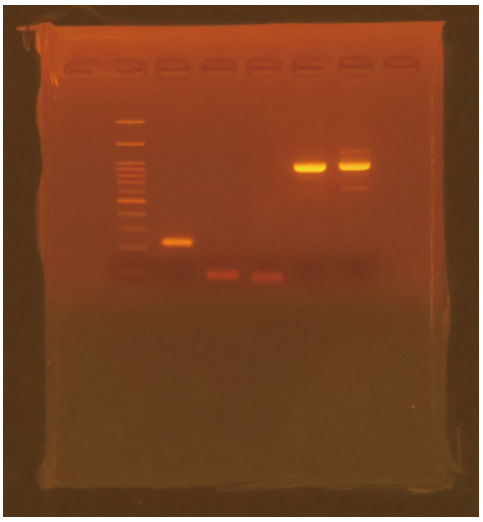
11) The amplified PCR product was extracted by phenol-chlorophorm. Precipitation and purification was carried out by ethanol
12) The PCR amplified DNA fragment was cloned into a vector
13) Size was confirmed by electrophoresis

● Electrophoretic conditions
100 V, 20 min
2 % agarose gel
1×TAE Buffer

14) Sequence was verified by sequencing

Result

M ① ② ③ ④ ⑤



← 1000 bp

← 250 bp

← 100 bp

M is a marker
The fragment of 100 bp (②,③) and 250 bp (①) is the UTR of the gene
The fragment of the 1000 bp (④,⑤) is the coding sequence

Customer comment

PCR amplified DNA fragement was cloned into a vector and the sequence was confirmed. As a result, there was no artificial mutation such as changes of single nucleotides.
Also, the manual of your product (FastGene® Scriptase II cDNA Synthesis Kit) was very easy to understand and I felt no stress on the operation.
I definitely want to use other products from NIPPON Genetics.

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