

Customers product feedback

Product name:	FastGene® Scriptase II cDNA Synthesis Kit (LS63)
Manufacture name:	Nippon Genetics Co., Ltd.
Application :	Cloning of isoflavone biosynthesis related enzyme using RNA extracted from soybene

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

Overview

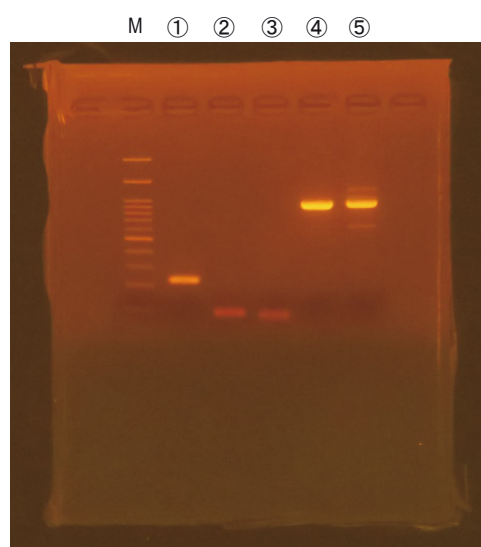
Plants adapt to changes in the enviroment by biosynthesizing specialized metabolites (secondary metabolites). In order to study the physiological functions of these substances, investigation of metabolic enzymes that biosynthesize them is essential. Here, we focused on soy isoflavone and conducted an 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	Protocol																							
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 	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 <table><tr><td>5x FastGene® Scriptase II buffer</td><td>4 µL</td></tr><tr><td>0.1 M DTT</td><td>2 µL</td></tr><tr><td>RNase Inhibitor</td><td>0.5 µL</td></tr></table> 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)	5x FastGene® Scriptase II buffer	4 µL	0.1 M DTT	2 µL	RNase Inhibitor	0.5 µL																	
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PCR reaction PCR enzyme : KOD FX Neo (TOYOBO) PCR Cycler : PCR Thermal Cycler Dice (TaKaRa)	10) PCR amplification of synthesized cDNA ● Reaction composition <table><tr><td>DW</td><td>10 µL</td></tr><tr><td>2x PCR Buffer for KOD FX Neo</td><td>25 µL</td></tr><tr><td>2 mM dNTPs</td><td>10 µL</td></tr><tr><td>Primer (10 µM each)</td><td>1.5 µL</td></tr><tr><td>Template (synthesized cDNA)</td><td>1 µL</td></tr><tr><td>KOD FX Neo (1 U/µL)</td><td>1 µL</td></tr><tr><td>Total</td><td>50 µL</td></tr></table> ● PCR program <table><tr><td>94°C</td><td>2 min (Initial denaturation)</td><td rowspan="4">30 cycles</td></tr><tr><td>↓</td><td>98°C 10 sec (Denaturation)</td></tr><tr><td>↓</td><td>55°C 30 sec (Annealing)</td></tr><tr><td>↓</td><td>68°C 1 min (Elongation)</td></tr></table>	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	94°C	2 min (Initial denaturation)	30 cycles	↓	98°C 10 sec (Denaturation)	↓	55°C 30 sec (Annealing)	↓	68°C 1 min (Elongation)
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Cloning Electrophoresis Gel imaging device : TF-20L (Vilber Lourmat) Nucleic acid staining reagent : Ethidium bromide	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																							
Sequencing	14) Sequence was verified by sequencing																							

Result



← 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's comments>

PCR amplified DNA fragment 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.