



## ■ 1. Experimental Materials and Methods

### 1.1 Instruments

MCE-202 MultiNA, PCR instrument

### 1.2 Reagents

Plant gene extraction reagent kit

(Beijing Kwinbon Biotechnology Co., Ltd.) FZ-002

SYBR® Premix Ex Taq™ II (Takara Bio Inc.) RR820A

SYBR® Gold Nucleic Acid Gel Stain (Invitrogen) S-11494

1× TE Buffer

25 bp DNA Ladder (Invitrogen) 10597-011

DNA-500 Reagent Kit for MultiNA

(Shimadzu Corporation) 292-27910-91

Samples: three groups of soybean and corn samples

Primers: According to the literature and the NCBI sequence, the primers designed for amplifying the soybean endogenous Lectin, corn endogenous Zein, exogenous promoter CaMV35S and exogenous terminator NOS genes are as shown in Table 1.

### 1.3 Analysis Conditions

DNA-500 on chip mode

### 1.4 Analysis Procedure

#### 1.4.1 DNA Extraction and Purification in Sample

**1.4.1.1** Grind about 1 g of plant tissues with liquid nitrogen, and transfer the powder to a 2 mL centrifuge tube.

**1.4.1.2** Add 0.5 mL of extract A, mix, and put in a 65 °C water bath for 1 hour.

**1.4.1.3** After bathing, add 1 mL of extract B: extract C=1:1 mixture to the tube, mix for 30 seconds, and then centrifuge at 12,000 rpm for 5 minutes.

**1.4.1.4** Pipette the upper aqueous phase into a new 2 mL centrifuge tube, add double volume of the pre-cooled anhydrous alcohol, 10 % volume of settling agent 1 and 4 μL of settling agent 2, mix, and allow to settle at -20 °C for 1 hour.

**1.4.1.5** After settlement, centrifuge at 12,000 rpm at 4 °C for 15 minutes, and pour away the supernatant carefully. At this point, white sediment can be seen at the bottom of the EP tube.

**1.4.1.6** Add 1 mL of pre-cooled washing solution, flip the EP tube to mix, centrifuge at 12,000 rpm at 4 °C for 5 minutes, then discard the supernatant, and invert the EP tube onto a filter paper for drying.

**1.4.1.7** Add 30 μL of dissolving solution to the dried EP tube for sediment dissolution, and maintain the resultant solution at -20 °C.

#### 1.4.2 PCR reaction system

See Tables 1 and 2 for the PCR reaction reagents and conditions.

Table 2 PCR Reaction Reagents

	Consumption	Final concentration
SYBR <sup>®</sup> Premix Ex Taq II (Tli RNaseH Plus) (2')	10.0 mL	1'
PCR Forward Primer (10 mM)	0.8 mL	0.4 mM
PCR Reverse Primer (10 mM)	0.8 mL	0.4 mM
DNA template	2.0 mL	20 ng/mL
dH <sub>2</sub> O (sterilized distilled water)	6.4 mL	
Total volume	20.0 mL	

Table 3 PCR Reaction Parameters

Action	Time/s	Temperature/°C
Activation and pre-denaturalization of DNA active enzyme	30	95
PCR (45 cycles)		
Denaturalization	30	95
Annealing	30	55
Extension	60	72
Post-cycle retention	180	72

### 1.4.3 MultiNA Determination

The PCR amplified products were subject to MultiNA determination. Based on the fragment size of the theoretical product, the 500 bp reagent kit was selected for determination in the experiment. In order to verify the accuracy of measurement, this paper also includes a negative control experiment, where the DNA template was not used.

## ■ Discussion of Results

Fig. 1 is the electropherogram of MultiNA measurement of the three groups of the soybean endogenous Lectin gene. The resultant fragments are amplified and clearly detected, and the fragment sizes are largely consistent with the expectation, indicating that the soybean genome has been extracted successfully. CaMV35S and NOS specific primers were used to amplify the genome of the three groups of soybean samples, and the results are as shown in Fig. 2 and Fig. 3. The results indicate that the expected CaMV35S 195 bp and NOS 180 bp bands were not detected, showing that all soybean samples were free from genetically modified CaMV35S and NOS.

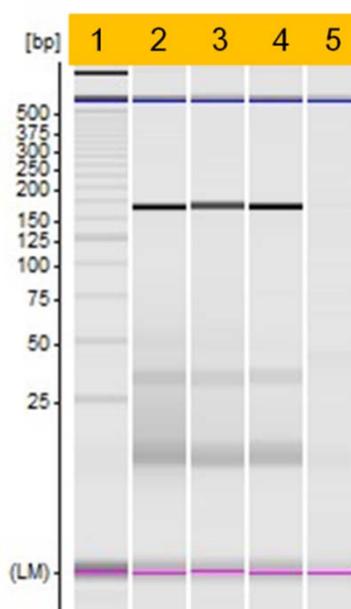


Fig. 1 Gel Determination Results of Soybean Endogenous Lectin (1: Ladder, 2: 1# soybean sample, 3: 2# soybean sample, 4: 3# soybean sample, 5: negative control)

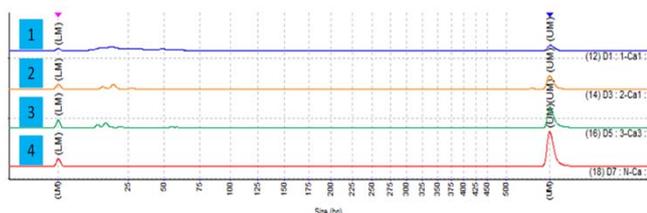


Fig. 2 Electrophoresis Determination Results of Soybean Exogenous Promoter CaMV35S (1: 1# soybean sample, 2: 2# soybean sample, 3: 3# soybean sample, 4: negative control)

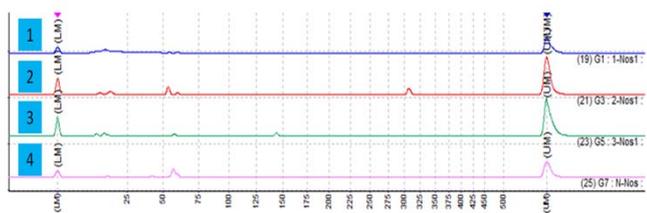


Fig. 3 Electrophoresis Determination Results of Soybean Exogenous Terminator NOS (1: 1# soybean sample, 2: 2# soybean sample, 3: 3# soybean sample, 4: negative control)

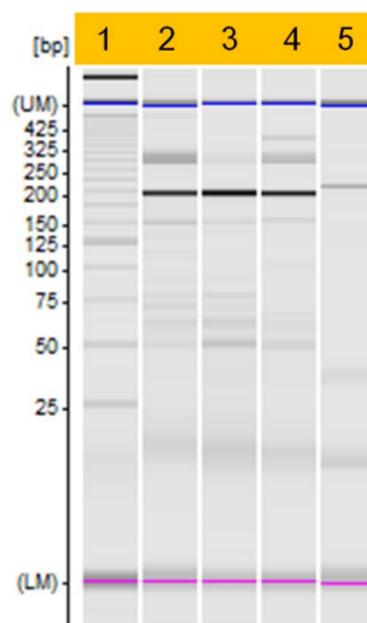


Fig. 4 Gel Determination Results of Corn Endogenous Zein (1: Ladder, 2: 1# corn sample, 3: 2# corn sample, 4: 3# corn sample, 5: negative control)

Fig. 4 is the electropherogram of MultiNA determination of the endogenous Zein gene in the three groups of corn samples. Similar to the soybean samples, the endogenous gene is detected clearly, indicating the successful extraction of the corn genome. Fig. 5 and Fig. 6 are the electropherograms of MultiNA determination of the PCR amplified products using CaMV35S and NOS as the target genes for primer design. Fig. 5 shows that no CaMV35S band with an expected length of 195 bp is amplified from the three groups of corn samples. Fig. 6 shows that 173 bp and 174 bp bands are amplified from the 1# and 2# corn samples, close to the theoretical NOS band of 180 bp, indicating that these two groups of corn samples may contain genetically modified ingredients. The promoter CaMV35S is not detected in these two groups of corn samples, possibly because this genetically modified corn does not contain the CaMV35S promoter or the genome of the samples is damaged to varying degrees.

### Conclusion

This paper establishes a method for the qualitative determination of genetically modified ingredients in crops using the Shimadzu MCE-202 MultiNA based on molecular biological technology. This method has high specificity and wide coverage for the determination of genetically modified ingredients, and can realize rapid transgene screening and determination, laying a good foundation for the subsequent identification of certain genes.

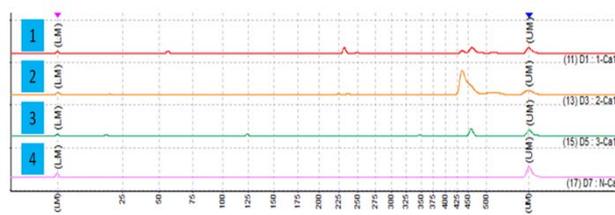


Fig. 5 Electrophoresis Determination Results of Corn Exogenous Promoter CaMV35S (1: 1# corn sample, 2: 2# corn sample, 3: 3# corn sample, 4: negative control)

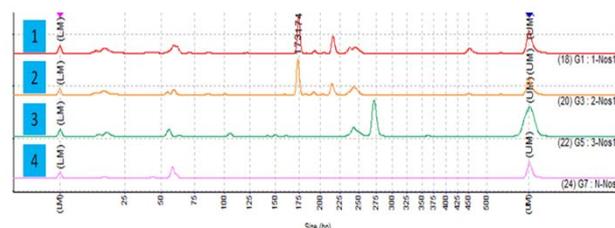


Fig. 6 Electrophoresis Determination Results of Corn Exogenous Terminator NOS (1: 1# corn sample, 2: 2# corn sample, 3: 3# corn sample, 4: negative control)