Detecting genetically modified organisms with the Agilent 2100 bioanalyzer

Application

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Abstract

Labeling of food containing more than 1% of genetically modified organisms (GMOs) has been obligatory in Europe since January 2000. To guarantee transparency and labeling, methods to distinguish between transgenic food and their traditional counterparts must be available. Genolife developed a method to detect Ready RoundUp soy (RRS) and a multiplex PCR to detect five corn transgenes (Bt176, Bt11, MON810, T25 and GA21). The Agilent 2100 bioanalyzer and DNA 500 LabChip® kit provided a simple, high throughput and standardized way to analyze multiplex PCR products. The methods developed by Genolife allow detecting 0.01% of RRS in food ingredients on the one hand and 10 copies of transgene MON810, GA21 and Bt11, and 100 copies of transgene Bt176 and T25 on the other.

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Introduction

The rapid development of biotechnology has launched products and ingredients derived from genetically modified organisms (GMOs) into the food market. The general public, however, has shown anxiety about this new technology. Information and transparency regarding these products are essential in order to become accepted by the consumers. In Europe, labeling of GMOs is regulated by the Novel Food directives 258/97/EEC[1] and 1139/98/EEC[2]. More recently, the "threshold regulation" 49/2000/EEC[3] has been approved, specifying that foodstuffs are subject to labeling. When the proportion of an individual food component is higher than 1 % manufacturers must label their products. Moreover, the presence of GMOs must be adventitious and therefore, food manufacturers must be able to supply evidence that they have taken appropriate steps to avoid using GMOs. A key factor to guarantee transparency and labeling is the availability of methods to distinguish between transgenic food and their traditional counterparts, not only in raw materials but also in food products.

Several analytical methods using polymerase chain reaction (PCR) technology have been developed to qualitatively detect the presence of a modified sequence of nucleic acid in transgenic food.[4,5] But these analytical methods detect only one modified sequence of one genetically modified organism. It would be advantageous to detect more than one sequence per genetically modified organism (one endogenous gene and several transgenic markers) or to screen several GMOs in one analysis. We therefore developed a method to detect RoundUp Ready soy (RRS) from Monsanto in one part, and another method to detect one endogenous maize gene and five genetically modified maize genes – four are authorized in Europe (Bt176, Bt11, MON810 and T25) and one is non-authorized (GA21). The Agilent 2100 bioanalyzer and DNA 500 LabChip® kit provided a simple, rapid and standardized alternative to analyze multiplex PCR products.

Results and discussion

RRS detection in food ingredients

DNA was extracted from commercial transgenic soybean reference standards (Fluka) and different food samples (lecithin, soybean proteins, soybean flour) with specific protocol developed by Genolife. For each sample, four PCR reactions were done — one for amplification of an endogenous gene (ACC1, 115 bp) to check the quality of the extracted DNA, and three PCR reactions for specific RR soybean sequences (T1: 167 bp, T2: 141 bp and T3: 189 bp). After amplification, PCR products were mixed and 1 µl of each mixed PCR was analyzed on the Agilent 2100 bioanalyzer using the DNA 500 LabChip kit, which allows analysis of DNA fragments ranging in size from 25 to 500 bp. Twelve samples were analyzed simultaneously and the 2100 bioanalyzer produced raw data and analysis in multiple formats. It displayed a simulated gel view an electropherogram. A data table labels each of the peaks and furnishes information about the size and concentration for each fragment. Results are shown in figure 1. Non transgenic soy gives

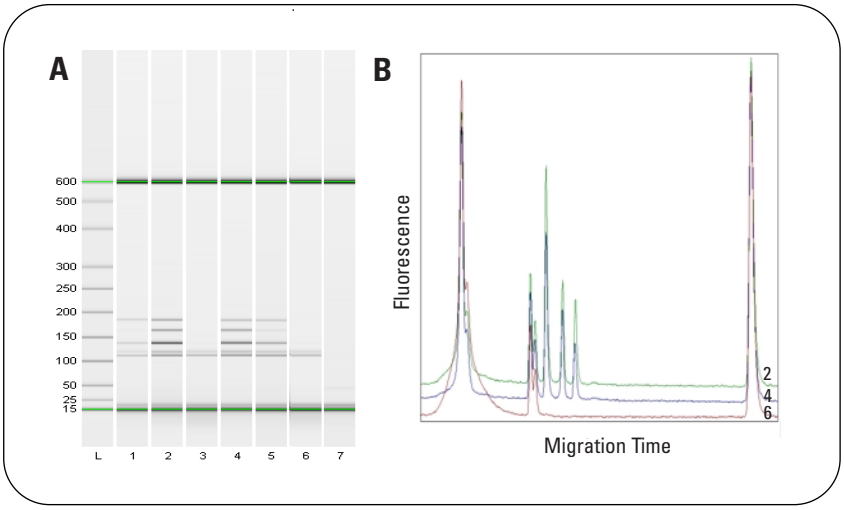

Figure 1
RR soybean detection
A) Gel view: 1-soya protein, 2- lecithin, 3-soybean flour, 4-RR soy 1 %, 5-RR soy 0.1 %, 6-non transgenic soy, 7-PCR blank.
B) Overlay of the electrophoretic traces of lanes 2, 4 and 6.

one band corresponding to the endogenous gene (115 bp) while RR soy 1 % and RR soy 0.1 % show four bands corresponding to the endogenous gene (115 bp) and the three specific RR soybean sequences (141, 167 and 189 bp). The Agilent 2100 bioanalyzer software compares unknown samples with commercial transgenic soybean reference standards. Soybean proteins and lecithin are transgenic (four bands at 115, 141, 167 and 189 bp) and soybean flour is not transgenic (only one band at 115 bp corresponding to endogenous gene). The 2100 bioanalyzer performs quantification using an internal standard (marker) added to each sample before loading, and the software calculates the DNA concentration in each band. Soybean protein contains less than 0.1 % and lecithin more than 1 % RR soy, compared to concentration DNA in transgenic band obtained with commercial transgenic soybean reference standards (table 1). The detection limit of this RR soy PCR is 0.01 %.

	RR1 %	RR 0.1 %	Soya protein	Lecithin
141 bp	1	0.24	0.12	1.3
167 bp	0.1	-	-	0.34
189 bp	1	0.56	0.48	1.3

Table 1
DNA concentration (ng/µl) of the band corresponding to the transgenic markers

GMO maize detection in food ingredients by multiplex PCR

In Europe, four GMOs maize were authorized — two insect-resistant corn species from Novartis (Bt11 and Bt176), one insect-resistant corn from Monsanto (MON810) and one glufosinate-tolerant corn developed by Agrevo (T25). We developed a PCR multiplex to detect these four corn lines and one endogenous gene to check the integrity of the extracted DNA. We also added a couple of primers to detect a glyphosate-tolerant corn GA21 produced by Monsanto. This glyphosate-tolerant corn is authorized in the USA and can be exported in Europe with authorized corn. The PCR multiplex amplified a 152-bp fragment for endogenous gene, a 343-bp fragment for Bt176 corn, a 149-bp fragment for T25, a 199-bp fragment for MON810, a 110-bp fragment for Bt11 and a 270-bp fragment for GA21. Results are presented in figure 2. The endogenous gene was amplified in all lanes except PCR blank. Only Bt176 fragment (343 bp) was obtained when only Bt176 corn was present in PCR tube (lane 4). Specificity was checked for each corn (lane 5: Bt11, lane 6: T25, lane 7: GA21 and lane 8: MON810). Lanes 1 to 3 presented PCR products when all corn lines were analyzed together. This multiplex PCR allows detection of five corn lines present at 0.2 % each (lane 3). The detection limit of this multiplex PCR is 10 copies for transgene MON810, GA21 and Bt11 and 100 copies of transgene Bt176 and T25.

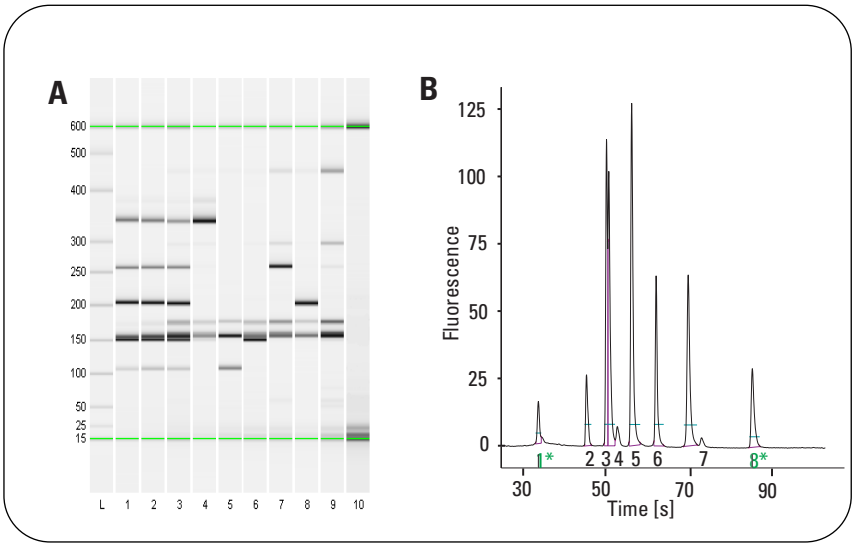

Figure 2
Multiplex PCR to detect GMO corn
A) 1-Bt176 5 %, Bt11 2 %, T25 5 %, GA21 5 %, MON810 5%, 2-Bt176 2 %, Bt11 2 %, T25 2 %, GA21 2 %, MON810 2 %, 3-Bt176 0.2 %, Bt11 0.2%, T25 0.2 %, GA21 0.3 %, MON810 0.3%, 4-Bt176 2 %, 5-Bt11 2 %, 6-T25 2 %, 7-GA21 2 %, 8-MON810 2 %, 9-non GMO corn, 10-PCR blanc
B) Electrophoregram of lane 2 (multiplex PCR with mix of 2 % corn). The peaks are: Bt11 corn (2), T25 corn (3), endogenous gene (4), MON810 corn (5), GA21 corn (6) and BT176 corn (7).

Conclusion

We developed detection methods for GMO soy and corn in food ingredients. The Agilent 2100 bioanalyzer performed the analysis of multiplex PCR product and allowed a semi quantification of GMO content. Two detection methods were presented— one for RR soy and one for GMO corn detection. The sensibility of the soya detection method is 0.01 % and 10 to 100 copies of transgene for corn mulitplex detection.

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