Application Note

Detection of genetically modified plants using the QIAxcel® System

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The QIAxcel System was successfully used to detect DNA derived from genetically modified organisms (GMOs) at a level suitable for GMO testing according to EU standards.

Introduction

According to current EU regulations that went into effect in 2004, food and feed that contain at least 0.9% content from GMOs, or that were derived from GMOs, must be labeled as such. Each GMO that is present must be specifically declared using the system of unique identifiers published by the EU. For products for which compliance can not be conclusively demonstrated by analysis (such as for highly refined materials), the non-GMO origin of the material must be documented. Enforcement of the regulation depends upon the availability of accurate methods for detection and tracing of GMO materials. This report describes the validation of a reliable and cost-effective system for GMO detection and analysis which facilitates compliance with EU guidelines.

The following performance characteristics were determined for GMO material mixed with non-modified plant material:

- DNA concentration
- Fragmentation state of DNA
- Specificity

The QIAxcel System. The fully automated size separation and quantification capability of the QIAxcel System provides unmatched resolution, speed, and throughput.

Sensitive GMO detection

To determine whether the limit of detection (LOD) of the QIAxcel System is sufficient for GMO testing, template DNA of GMO origin (35S promoter) was mixed with genomic DNA from non-modified plants. Amplification of the GMO band was detected using the QIAxcel System (Figure 1) and quantification was performed to determine the sensitivity of detection (Figure 2 and Table 1).

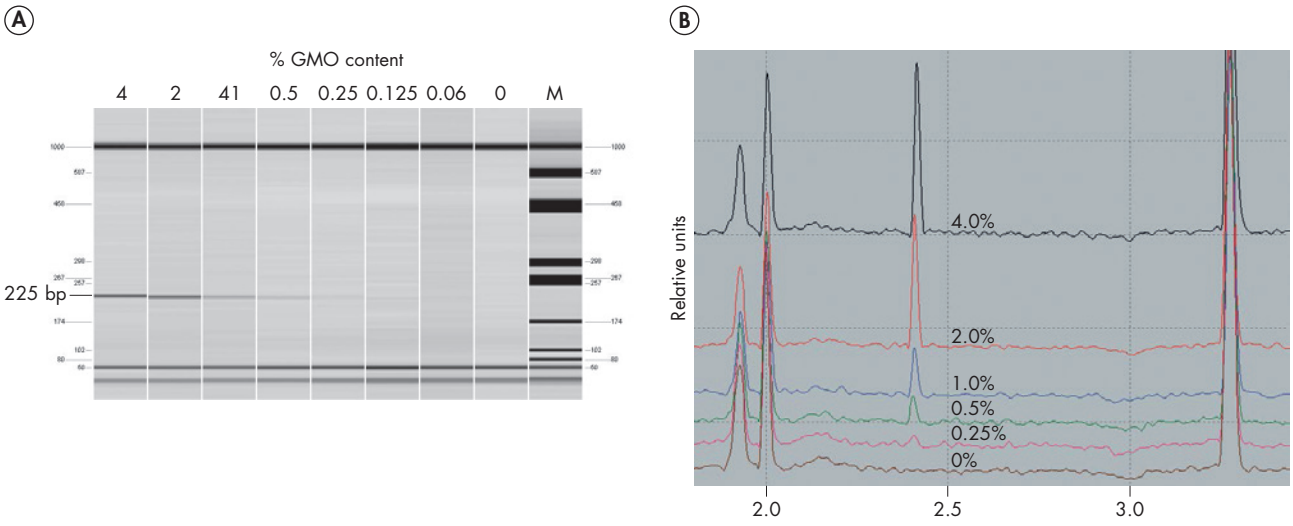

Figure 1. Amplification of target DNA mixed with genomic DNA from non-modified plants. Genomic DNA (supplied by University of Tennesee) was purified from young dogwood leaves using the DNeasy® Plant Mini Kit and quantified with a TD-360 fluorometer (Turner Designs, Sunnyvale, USA). The integrity of the genomic DNA was examined by visual inspection on a 1% agarose gel. Control 35S DNA template (provided by GeneScan, Freiberg, Germany) was mixed with the plant genomic DNA to the final concentrations listed in Table 1. Reactions were prepared with 50 ng/µl of the genomic DNA mixtures and proprietary primers (provided by Biotools, Madrid, Spain). PCR was performed on an Mastercycler® Gradient (Eppendorf, Hamburg, Germany): after an initial denaturation step (94°C for 3 minutes), 40 PCR cycles (denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 40 seconds) were followed by 1 step of 72°C for 3 minutes. PCR products were analyzed in the automatic multi-capillary electrophoresis QIAxcel System using the QIAxcel DNA Screening Kit. Non-diluted PCR products were placed in the instrument sample tray. The DNA samples were automatically injected into the capillary channel and subjected to electrophoresis according to the standard protocol. Separation was performed by the AM320 method using 10 seconds injection time and 320 seconds separation time. **A.** The expected 225 bp band amplified from the 35S promoter is clearly visible in the gel image. **M**: The molecular weight and concentration of the amplicons were determined based on the standard pUC18/HaeIII DNA size marker (Sigma, St. Louis, USA). **B.** BioCalculator software labeled the integrated peak area automatically. The added 35S target DNA was detected at a level of 0.25% in the electropherogram.

Table 1. Detection of GMO content

Template copies	GMO content (%)	Detected
2000	4	Yes
1000	2	Yes
500	1	Yes
250	0.5	Yes
125	0.25	Yes
62	0.125	No
31	0.06	No
No template control	0.0	No

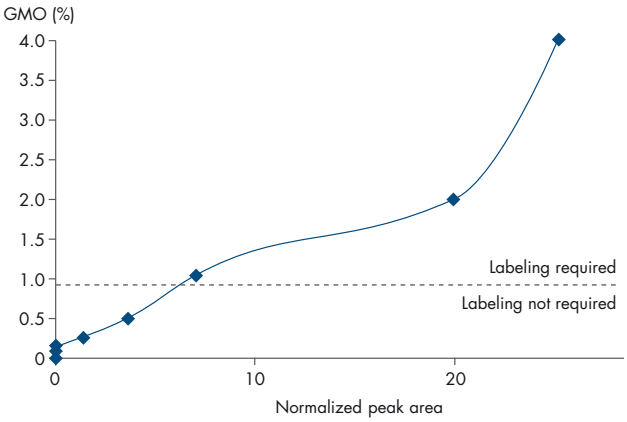

Figure 2. LOD of the QIAxcel System. The LOD was calculated for the quantitative normalized area (peak area) of the amplified 35S product at a defined number of copies per reaction. According to the EU GMO regulation, the limit of detection for a GMO marker must be below 0.9%. Food containing over 0.9% GMO content must be labeled.

Conclusions

- This system detected as few as 125 copies of GMO-origin target mixed with 50 ng genomic DNA from non-modified plants, corresponding to 0.25% GMO content (see Table 1). This result indicates that the sensitivity of the system allows detection of GMO content well below the cutoff limit for labeling (0.9%).
- The QIAxcel System includes a consumable multi-channel cartridge that can be used to inject and analyze multiple DNA samples simultaneously: up to 12 samples can be analyzed in less than 7 minutes and 96 samples in a multiwell plate in less than 50 minutes. This throughput is sufficient to meet the needs of many GMO-testing facilities.

QIAxcel Kits. Precast, reusable gel cartridges allow up to 200 runs of 12 samples to be performed.

Reference

Liu M.S. and Amirkhanian V.D. (2003) DNA fragment analysis by an affordable multiple-channel capillary electrophoresis system. Electrophoresis **24**, 93.0.

Ordering Information

Product	Contents	Cat. no.
QIAxcel System	Capillary electrophoresis device, including computer, and BioCalculator Analysis software; 1-year warranty on parts and labor	9001421
Warranty PLUS 2 Basic, QIAxcel	3-year warranty, 5-working day response time, all labor, travel, and repair parts	9241202
Gel cartridge kits		
QIAxcel DNA High Resolution Kit (1200)*	QIAxcel DNA High Resolution Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QIAxcel DNA Screening Kit (2400)*	QIAxcel DNA Screening Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929004
QIAxcel DNA Large Fragment Kit (600)*	QIAxcel DNA Large Fragment Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929006
Software		
BioCalculator Software†	Separate license for use of BioCalculator software on an additional computer	9018391
Purification of plant genomic DNA		
DNeasy Plant Mini Kit (50)	50 DNeasy Mini Spin Columns, 50 QIAshredder Mini Spin Columns, RNase A, Buffers, Collection Tubes (2 ml)	69104
DNeasy Plant Mini Kit (250)	250 DNeasy Mini Spin Columns, 250 QIAshredder Mini Spin Columns, RNase A, Buffers, Collection Tubes (2 ml)	69106

* QX DNA Size Markers and QX Alignment Markers are not provided with QIAxcel DNA Kits and must be ordered separately. For information about markers suitable for your application, visit **www.qiagen.com/QIAxcel**.

† The software key is for analysis of results only. It does not provide any instrument control functions.

The QIAxcel instrument is intended to be used only in combination with QIAxcel Kits for applications described in the respective QIAxcel Kit handbooks. QIAxcel Kits are intended for research only. Not for use in diagnostic procedures.

The DNeasy Plant Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention or treatment of a disease.

Discover more about how the QIAxcel system can speed up and increase sensitivity for GMO detection at **www.qiagen.com/goto/QIAxcel**.