# Identifying allergenic nut species using the QIAxcel® system

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## Introduction

The global food industry faces numerous challenges in terms of providing confidence in the authenticity of foods. There is a commercial interest in ensuring that foods are correctly labeled for the presence and levels of specific ingredients, including the occurrence of GMOs and incidence of allergens, and in preventing the fraudulent replacement of expensive food ingredients with inferior ones.

For the benefit of consumers who are allergic to certain food ingredients, current EU regulations require labeling disclosure of the presence of any of 14 major allergens if used as ingredients in prepacked foods (Directive 2003/89/EC, as amended). PCR assays have been developed to detect and identify DNA originating from allergenic nut materials in processed foods. They consisted of a suite of singleplex and multiplex assays to detect DNA from almond, hazelnut, macadamia nut, and peanut. The assays have been validated by LGC (UK) and Premier Analytical Services (UK) with regards to sensitivity and specificity.

This study demonstrates the successful use of the QIAxcel system for the analysis of PCR products as part of an allergen detection workflow. The QIAxcel system provided rapid, reliable, and inexpensive identification of four nut species.

## Materials and methods

Samples from a panel of 17 food products (Table 1) were analyzed using singleplex and multiplex PCR assays developed for the nut species almond, hazelnut, macadamia nut, and peanut. The PCR products were analyzed using the QIAxcel system in combination with the QIAxcel DNA High Resolution Kit.

End-point PCR was conducted for each nut species using 200 µM primers and 50 ng template DNA in a final volume of 25 µl. The PCR products were in the size range 75 to 134 bp. Short fragment sizes were chosen to ensure that the targets remained intact during food processing. The DNA concentration of the samples tested was in the range 2–5 ng/µl.

Samples were analyzed using a QIAxcel DNA High Resolution Cartridge. The method designated OM500 was chosen as the optimal one for the nut species under analysis. It has the following parameters: QX Alignment Marker injection at 4 kV and 20 s, sample injection at 5 kV for 10 s and separation at 5 kV for 500 s. The sample injection time was increased to 40 s to ensure ▶

identification of all of the amplicons present in the sample. The analysis was performed with a suspend integration time of 2.53 min. QX Alignment Marker 15 bp/400 bp and QX DNA Size Marker 25–500 bp were run simultaneously. Two fragments from the QX DNA Size Marker were excluded from the analysis since they were longer than the 400 bp fragment in the QX Alignment Marker.

## Results

The amplicons from the singleplex and multiplex PCR assays were analyzed using the QIAxcel system and QIAxcel DNA High Resolution Kit (Figure 1). An overview of the assays and the fragments detected is presented in Table 1. All of the nuts present on the ingredients list of the samples were accurately identified. An electropherogram of sample 16 is also presented to illustrate the successful application of the newly developed PCR method (Figure 2). The limit of detection for all of the nut species based on singleplex assays using 100% nut material was less than 20 pg of nut DNA.

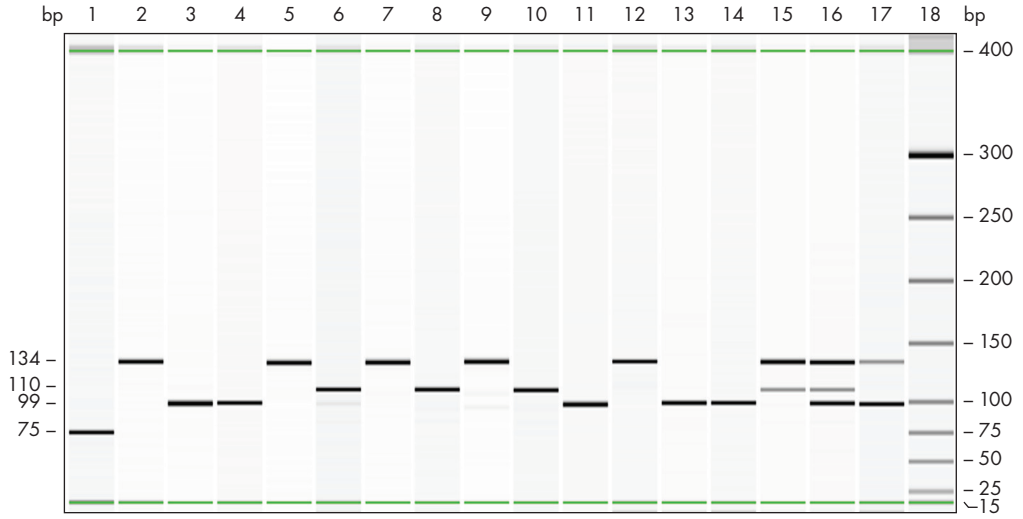


**Figure 1. Successful use of the QIAxcel system for DNA fragment analysis.** DNA fragments from macadamia, peanut, hazelnut, and almond are clearly visible. **Lane 1**: Macadamia (75 bp); **lanes 3, 4, 11, 13, 14, 16, and 17**: peanut (99 bp); **lanes 6, 8, 10, 15 and 16**: hazelnut (110 bp); **lanes 2, 5, 7, 9, 12, 15, 16, and 17**: almond (134 bp); **lane 18**: QX DNA Size Marker 25–500 bp.

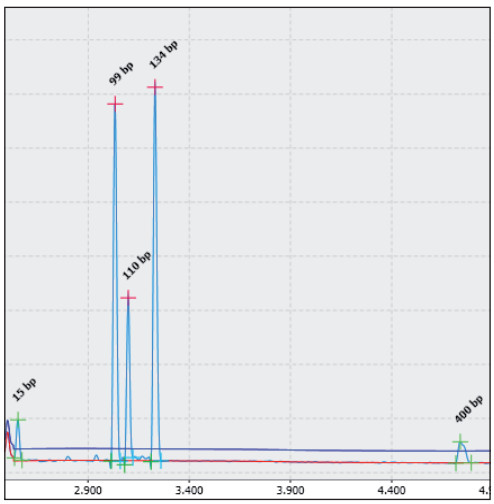


**Figure 2. Electropherogram displaying the result from the multiplex PCR, sample 16 (cereal bar).** DNA fragments from peanut (99 bp), hazelnut (110 bp), and almond (134 bp) were detected. The ingredients listed in the cereal bar included almonds (15%), peanuts (15%), and hazelnuts (9%).

**Table 1. Foods tested for their nut contents with singleplex and multiplex assays using the QIAxcel system**

| Food sample | Lane | Nuts present on ingredient list | Assay | Fragment(s) detected (bp) |
|---|---|---|---|---|
| Macadamia cookies | 1 | Macadamia | Macadamia | 75 |
| Marzipan | 2 | Almond | Almond | 134 |
| Peanut butter | 3 | Peanut | Peanut | 99 |
| Brownies | 4 | May contain nuts | Peanut | 99 |
| Pistachio baklawa | 5 | Bakery (Pistachio) | Almond | 133 |
| Pistachio baklawa | 6 | Bakery (Pistachio) | Hazelnut | 110 |
| Dark chocolate | 7 | Almond, hazelnut | Almond | 133 |
| Dark chocolate | 8 | Almond, hazelnut | Hazelnut | 110 |
| Cereal bars | 9 | Almond, peanut, hazelnut | Almond | 134 |
| Cereal bars | 10 | Almond, peanut, hazelnut | Hazelnut | 110 |
| Cereal bars | 11 | Hazelnut | Peanut | 98 |
| Marzipan | 12 | Almond | Multiplex | 134 |
| Peanut butter | 13 | Peanut | Multiplex | 99 |
| Brownies | 14 | May contain nuts | Multiplex | 99 |
| Dark chocolate | 15 | Almond and hazelnut | Multiplex | 110/134 |
| Cereal bars | 16 | Almond, peanut, hazelnut | Multiplex | 99/110/134 |
| Pistachio baklawa | 17 | Bakery (Pistachio) | Multiplex | 98/134 |

Detecting the singleplex and multiplex nut assays and analyzing the data using the QIAxcel ScreenGel® software demonstrated that the QIAxcel system can be used to detect DNA of specific nut species in the foods tested. The specific assays yielded clear results with strong bands corresponding to the targeted fragment sizes.

The results of the analysis can also be presented in other formats.

1. A table with up to 16 user-defined parameters, including size, concentration, and percentage.
2. Peak calling tables with information about the presence or absence, size, and concentration of the targeted amplicons in the analyzed samples

## Conclusions

The QIAxcel system can be successfully used to identify PCR products from the DNA of the specific nut varieties almond, hazelnut, macadamia nut, and peanut. Thanks to the fully automated procedure, the results are highly reproducible and reliable. Safety and reproducibility is ensured by defining the process profile, which cannot be changed by a routine user. The process profile defines the electrophoresis parameters, analysis, and reporting of the data.

This method used with the QIAxcel DNA High Resolution Kit and QIAxcel system proved to be an excellent tool for identifying nut DNA. These results demonstrate the successful application of the PCR method across a range of food samples and also the high sizing accuracy and reproducibility of results yielded by the QIAxcel electrophoresis system.

**Acknowledgements**

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**References**

1. Burrell, A., Foy, C. and Burns, M. (2011) Applicability of Three Alternative Instruments for Food Authenticity Analysis: GMO Identification. Biotechnology Research International doi:10.4061/2011/838232
2. Valdivia, H. and Burns, M. (2010) Sample Preparation and DNA extraction for the Detection of Allergenic Nut Materials. Journal of the Association of Public Analysts (Online) **38**, 01

## Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| QIAxcel Advanced System | Capillary electrophoresis device: includes computer, software, and 1-year warranty on parts and labor | 9001941 |
| QIAxcel DNA High Resolution Kit (1200) | QIAxcel DNA High Resolution Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips | 929002 |
| QX Alignment Marker 15 bp/400 bp | Alignment marker with 15 bp and 400 kb fragments | 929521 |
| QX DNA Size Marker 25–500 bp (50 µl) v2.0 | DNA size marker with fragments of 25, 50, 75, 100, 150, 200, 250, 300, 400, and 500 bp; concentration 100 ng/µl | 929563 |

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