

Species determination for meat using PCR-RFLP analysis on the QIAxcel[®] system

Christoph Graf,¹ Agne Rüegg-Kuslyte,² and Roger Kuhn²

¹ Official Regulatory Authority for Food of Canton Bern, Bern, Switzerland

² Department of Life Science and Facility Management, Zurich University of Applied Sciences (ZHAW), Wädenswil, Switzerland

A strategy using PCR followed by restriction fragment length polymorphism analysis (PCR-RFLP) is used to identify meat from various animal species in food. Analysis results from the QIAxcel system and from agarose gel electrophoresis were compared for 14 exotic and game species. Although both techniques were suitable for species differentiation, the use of the QIAxcel was less time consuming and provided the advantage of electronic documentation.

Introduction

Today, meats from exotic and game animals from all around the world provide an attractive alternative for people looking for unforgettable sensory experiences. Falsification of specialty meat is very common due to the tremendous profit gained by selling less costly meat labeled as meat from a more expensive species. A method that is sensitive enough to detect the small but relevant difference between meat from specific species is essential for official food control authorities to verify claims made about specialty meat. Protein-based methods for species identification, such as isoelectric focusing (IEF) or immunological methods, are not adequate because the soluble muscle proteins in processed meat products (heated or marinated) are rapidly and efficiently degraded. Nucleic acid-based analytical methods for the differentiation and identification of animal species in food have been commonly used in the last 20 years (1, 2).

In this study, PCR amplification of the cytochrome b (cytb) gene followed by restriction fragment length polymorphism analysis (PCR-RFLP) was used to differentiate 14 different exotic or game species. Traditionally, gel electrophoresis has been used to detect PCR-RFLP DNA fragments. However, this method is laborious, time consuming, and hazardous due to the use of ethidium bromide or similar intercalating dyes that are mutagenic and dangerous for human health. In addition, gel data can not be used directly for publication or archiving. As an alternative method for discriminating animal species in processed food and meat, we evaluated the QIAxcel capillary electrophoresis system, a computer-controlled system that provides electronic documentation.

Materials and Methods

Samples were collected and total RNA/DNA was extracted using the QIAamp® Viral RNA Mini Kit. A two-tube multiplex reverse-transcription PCR assay (two-tube assay) was used to detect 16 respiratory viruses based on their amplicon size differences.

Nucleic acid purification

DNA extraction was performed using the Wizard Plus Miniprep® DNA Purification System (Promega) according to the *Swiss Food Manual* (4). DNA was extracted from 200 mg ground meat from bison, chamois, crocodile, duck, emu, kangaroo, kudu, ostrich, quail, rabbit, red deer, roe deer, springbok, and water buffalo and was eluted in 50 µl of elution buffer according to the manual.

PCR-RFLP analysis

Primers to conserved regions of the vertebrate mitochondrial cytb gene were used to amplify a 359 bp fragment (1, 3, 5). PCR amplification of 25 µl reactions, as well as restriction analysis, was performed as described in (5). PCR-RFLP products were separated on 3% agarose gels in TAE

buffer, and the BenchTop 100 bp DNA Ladder (Promega) was included in the analysis. In addition, analysis was performed on the QIAxcel system using the QIAxcel DNA High Resolution Kit with the OM700 method. The QX Alignment Marker 15 bp/1 kb was included in the analysis.

Results

Using a set of endonucleases, all species could be identified with both conventional agarose gel electrophoresis and the QIAxcel capillary electrophoresis system. Using the QIAxcel system, 24 samples were analyzed in approximately 30 minutes. Analysis using agarose gel electrophoresis, which involves more steps for handling and documentation, required at least three times as long.

Representative analysis results are shown in Figures 1 and 2. Fragments shorter than 100 bp were not visible on the agarose gel. A 35 bp fragment from samples 11 and 12 (Figure 1) and fragments between 50 bp and 80 bp from samples 11, 12, 14, and 15 (Figure 2) were detected using the QIAxcel system but not with agarose gel electrophoresis.

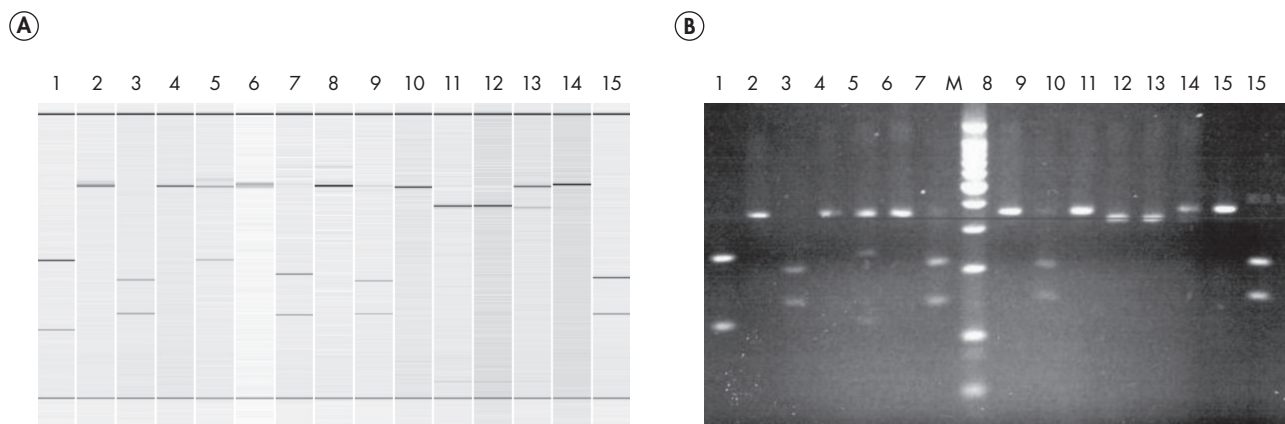


Figure 1. PCR-RFLP analysis (*RsaI*) of meat from several exotic and game species. DNA isolated from crocodile (1), kangaroo (2), ostrich (3), duck (4), red deer (5), roe deer (6), kudu (7), springbok (8), quail (9), rabbit (10), bison (11, 12); water buffalo (13), chamois (14), and emu (15) was amplified by PCR, digested with *RsaI* and analyzed using **A.** the QIAxcel system with the QIAxcel DNA High Resolution Kit or **B.** conventional agarose gel electrophoresis. **M:** 100 bp DNA Ladder.

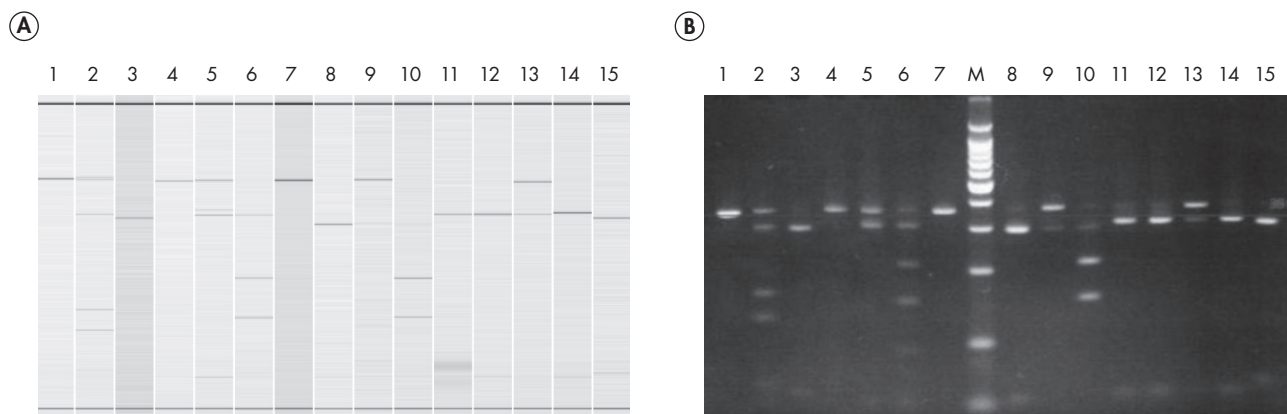


Figure 2. PCR-RFLP analysis (*Tru9 I*) of meat from several exotic and game species. DNA isolated from crocodile (1), kangaroo (2), ostrich (3), duck (4), red deer (5), roe deer (6), kudu (7), springbok (8), quail (9), rabbit (10), bison (11, 12); water buffalo (13), chamois (14), and emu (15) was amplified by PCR, digested with *Tru9 I* and analyzed using **A.** the QIAxcel system with the QIAxcel DNA High Resolution Kit or **B.** traditional agarose gel electrophoresis. **M:** 100 bp DNA Ladder.

Conclusions

- The QIAxcel capillary electrophoresis system as well as conventional agarose gel electrophoresis provided suitable differentiation of relevant animal species. However, compared to agarose gel electrophoresis, QIAxcel analysis enables significantly shorter running time, eliminates sample preparation and exposure to mutagenic reagents, and requires fewer analysis and handling steps, freeing time for more demanding laboratory work and reducing manual errors.
- Fragments shorter than 100 bp were detected with the QIAxcel system but not with agarose gels, greatly reducing the practical value of the agarose gel system.
- QIAxcel documentation of results, presented as a gel-like image, an electropherogram, or in table format, is automatic and reliable. The QIAxcel system provides more detailed information than conventional agarose gel electrophoresis.

References

1. Meyer, R., Höfelein, C., Lüthi, J., and Candrian, U. (1995) Polymerase chain reaction-restriction fragment length polymorphism analysis: a simple method for species identification in food. *JAOAC Int.* **78**, 1542.
2. Matsumura, T.K., Chikuni, K., Tanabe, R., Muroya, S., Shitaba, K., Yamada, J., and Shinmura, Y. (1999) A quick and simple method for the identification of meat species and meat products by PCR assay. *Meat Sci.* **51**, 143.
3. Wolf, C., Rentsch, J., and Hubner, P. (1999) PCR-RFLP analysis of mitochondrial DNA: a reliable method for species identification. *J. Agric. Food Chem.* **47**, 1350.
4. *Schweizerisches Lebensmittelbuch SLMB* (Swiss Food Manual SLMB) www.slmb.admin.ch/slmb/index.html.
5. Maede, D. (2006) A strategy for molecular species detection in meat and meat products by PCR-RFLP and DNA sequencing using mitochondrial and chromosomal genetic sequences. *Euro. Food Res. Technol.* **224**, 209.

Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced System	Capillary electrophoresis device, including computer, and QIAxcel ScreenGel Software; 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
QIAxcel DNA Screening Kit (2400)	QIAxcel DNA Screening Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929004
QIAxcel DNA Fast Analysis Kit (3000)	QIAxcel DNA Fast Analysis Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX DNA Size Marker 50 bp – 1.5 kb, QX Alignment Marker 15 bp/3 kb, 12-Tube Strips	929008
QIAxcel RNA QC Kit v2.0 (1200)	For 100 runs of 12 samples: QIAxcel RNA Quality Control Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX RNA Alignment Marker, QX RNA Size Marker 200–6000 nt, QX RNA Denaturation Buffer, 12-Tube Strips	929104

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