Meat species identification using PCR-RFLP and native capillary electrophoresis system

Mirjana Kozulic[1] and Renaud Cassier[2]

[1]QIAGEN Instruments AG, CH-8634 Hombrechtikon, Switzerland; [2]ADGENE Laboratoire, Thury Harcourt, France

Introduction: A growing need for reliable meat species identification

- In order to protect customers' interests, it has been necessary to develop effective methods that enable verification of the species composition of different food products.
- In EU countries, producers must ensure that production processes comply with European Council (EC) Regulation No. 510/2006 and that their products are correctly labeled in compliance with EC Regulation No. 13/2000.
- Reliable meat species identification is important to prevent incorrect handling, including fraud and unintentional mixups, which may lead to health problems and/or violation of religious beliefs.
- Most methods for meat species identification are PCR-based, since identification is possible in raw and processed foods.
- The purpose of this study was to optimize the well-known method of PCR-RFLP. The process was optimized in conjunction with the QIAxcel® Advanced System.
- The optimized method delivers results in under 8 hours and is highly suited to routine analyses.

Currently available methods

- The most commonly used methods are as follows.

Methods	Advantages	Limitations	References
PCR with species-specific primers on cytochrome B mitochondrial DNA	■ Simple method ■ Processed meat can be sampled ■ Multiplexing possible	■ Only a few species can be detected ■ Can only be used for detection of pre-specified species	Colombo, et al. 2002 Schwägele, et al. 2007
Real-time PCR	■ Can detect trace amounts ■ Processed meat can be sampled ■ Quantitative (DNA) analysis ■ Multiplexing possible	■ Can only be used for detection of pre-specified species ■ Costs can be very high when searching for several animals	Tanabe, et al. 2007 Pegels, et al. 2011
PCR-RFLP on a specific region of the mitochondrial genome (tRNAGlu/ cytochrome b)	■ One analysis for detection and identification of up to 25 animals ■ Processed meat can be sampled ■ Specific and sensitive	■ Point mutations can yield false-negative results ■ A database is necessary ■ No automated electrophoresis; analysis is time-consuming	Wolf, et al. 1999 Pascoal, et al. 2004 Maede, 2006

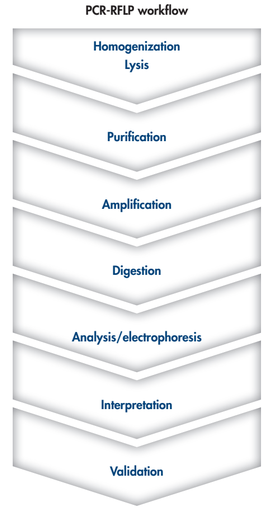
- Most PCR methods require mitochondrial DNA. Hundreds to thousands of copies of mtDNA are present in each animal cell. These numerous copies increase the chance of detecting the target animal DNA, even in processed food (Pascoal, et al. 2011, Maede, 2006).
- DNA detection in processed food can be very difficult because intensive treatment of food can degrade and fragment DNA. It is therefore crucial to use short amplicons (<500 bp).

The QIAxcel based PCR-RFLP protocol

We have optimized and validated an efficient PCR-RFLP protocol for semi-automated meat species identification.

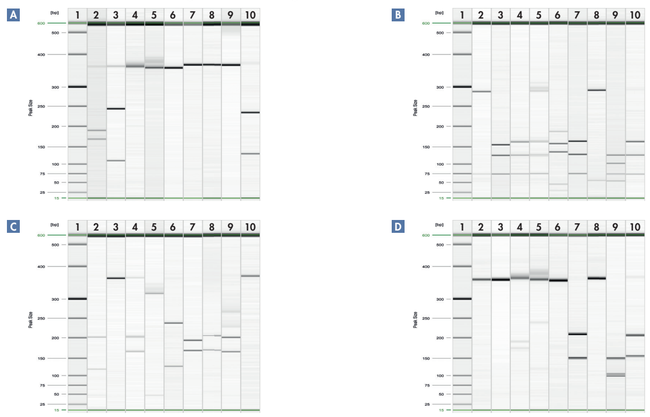
Protocol in brief

1. Homogenization and lysis of meat samples from various animal species of interest (cattle, pig, sheep, deer, rabbit, chicken, duck, turkey, and goose).
2. DNA purification using QIAsymphony® and the QIAsymphony DSP DNA Mini Kit.
3. PCR amplification of the DNA samples.
4. Digestion of the PCR products with 4 restriction enzymes (*Alu*I, *Hae*III, *Hin*fI and *Rsa*I)
5. Analysis of digested samples by native capillary electrophoresis using the QIAxcel Advanced System.
6. Interpretation of the results using a programmed Excel® sheet.
7. Validation using QIAxcel ScreenGel software.



Digestion scheme: Identification of meat species

The data below show the results from a study of 9 animal species. For validation, the samples were analyzed by real-time PCR and the results compared to data obtained by PCR-RFLP following analysis using QIAxcel ScreenGel software. The two methods gave 100% congruent results.



Restriction digestion. Fragments after 2 hours' digestion of the 359 bp mitochondrial cytb fragment with restriction enzymes. **A** *Alu*I. **B** *Hae*III. **C** *Hin*fI. **D** *Rsa*I. Lanes 1: Size marker; 2: Cattle; 3: Pig; 4: Sheep; 5: Deer; 6: Rabbit; 7: Chicken; 8: Duck; 9: Turkey; 10: Goose.

Analysis of commercial meat samples

Commercial meat samples were analyzed using PCR-RFLP. The data obtained were compared with the product labels. The gel image is an example of how the QIAxcel Advanced System displays results. The time-saving, semi-automated method enables reliable meat species identification on a large scale, regardless of whether the meat is raw, frozen, cooked, dehydrated, sterilized, or smoked.

QIAxcel Advanced analysis of 6 commercial samples. FF1: pork pâté (100% pig); FF2: hamburger meat (100% beef); FF3: Parmentier beef hash (100% beef); FF4: pork terrine (100% pig); FF5: pork liver mousse (100% pig); FF6: poultry liver terrine (75% pig and 25% poultry). These samples were digested by the following enzymes. Lanes A1-A5: *Alu*I; Lanes: C2-C7: *Hin*fI; Lanes: D3-D8: *Hae*III; Lanes: E3-E: *Rsa*I.

Sample	Expected result	Observed result	Compliance
FF1 (pork pâté)	Pig	Pig	Yes
FF2 (Hamburger meat)	Beef (cattle)	Beef + Pig	No
FF3 (Parmentier beef hash)	Beef	Beef + Pig (traces)	No
FF4 (Pork terrine)	Pig	Pig	Yes
FF5 (Pork liver mousse)	Pig	Pig	Yes
FF6 (Poultry liver terrine)	Pig + Poultry	Pig + Chicken	Yes

Summary of sample analysis . Two samples (FF2 and FF3) were mislabeled. Some pig DNA was detected where only beef was expected.

Conclusion

- Compared with other methods, the PCR-RFLP protocol in combination with the QIAxcel Advanced System is a powerful tool; it enables discrimination of a large number of meat species on a large scale and with high sensitivity in one working day (8 hours).
- QIAxcel Advanced eliminates exposure to hazardous chemicals, such as ethidium bromide, providing a safer method for the user.
- The semi-automated method is reliable and affordable, therefore a good candidate for use in the food industry.
- Potential procedural or interpretation errors are reduced through semi-automation, provided that the database is complete and handled correctly.

References

Colombo, F et al. (2002) Meat Sci. **61**, 291. Maede, D. (2006) Eur. Food Res. Technol. **224**, 209. Pascoal, A. et al. (2004) Eur. Food Res. Technol. **218**, 306. Pegels, N, et al. (2011) Food Control **22**, 1189. Schwägele, F, et al. (2005) Meat Sci. **71**, 164. Tanabe, S, et al. (2007) Biosci. Biotechnol. Biochem. **71**, 3131. Wolf, C, et al, (1999) J. Agric. Food Chem. **47**, 1350.

The applications presented here are for molecular biology applications. They are not intended for the diagnosis, prevention or treatment of a disease.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

1083078 07/2014