Species testing using PCR

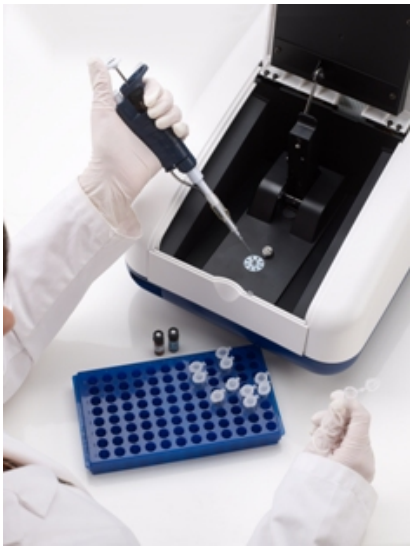
Introduction

Widespread reports of food adulteration and mislabelling at the beginning of 2013 highlighted major failures in food traceability, with numerous cases of horsemeat contamination reported to have been found in a range of processed food products.

Many commercial kits are available for identification of animal species in mixed samples, many of which are based on PCR technology. We demonstrate here the use of the Techne Prime thermal cycler for species identification using DNA extracted from various animal sources including horse, beef and pork. The results indicated that whole cuts of meat were species-pure but minced products were prone to contamination.

Methods

DNA from samples of horse hair and mouth swabs, minced beef and pork fillet were extracted using the DNeasy® Blood and Tissue kit (Qiagen, part code 69504). Four extractions were made from each meat sample and two from each of horse hair and horse mouth swabs. Samples were eluted from the spin columns with 200µl of elution buffer. The yield of DNA was measured for each extraction using the Jenway Genova Nano micro volume spectrophotometer[1]. 2µl of sample were placed on the read head and the concentration determined using the dsDNA mode with 320nm correction and a factor of 50 for dsDNA. Values are shown in Table 1.



Sample	Absorbance at 260nm	Concentration (µg/ml)
Horse 1 (hair)	0.042	41.51
Horse 2 (hair)	0.036	35.86
Horse 3 (swab)	0.012	12.28
Horse 4 (swab)	0.014	14.29
Pork 1	0.059	59.17
Pork 2	0.079	78.50
Pork 3	0.092	91.74
Pork 4	0.058	57.66
Beef 1	0.066	65.89
Beef 2	0.069	69.16
Beef 3	0.066	66.26
Beef 4	0.072	72.21

Table 1: DNA concentration of extracted samples measured using the Jenway Genova Nano micro volume spectrophotometer. Values are the average of two determinations.

Species testing kits for conventional PCR were obtained from Genekam Biotechnology AG (http://www.genekam.de/en/). The kits used were as follows:

K024 Bovine DNA Identification.
K029 PCR Identification of Porcine DNA.
K603 PCR Identification of Equine (Horse) DNA.

[1] http://www.jenway.com/product.asp?dsl=885

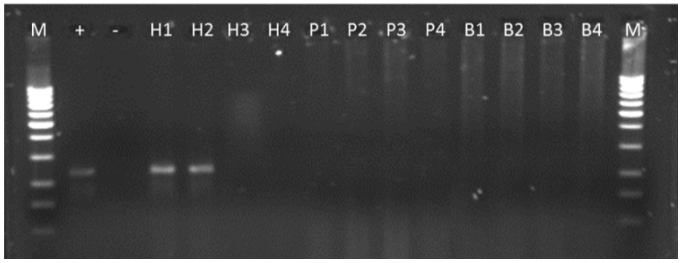
All samples were tested with each of the kits following the manufacturer's instructions. Thermal cycling was performed in a Techne Prime thermal cycler[2]. Following amplification, 10µl of each reaction were run on 2% agarose gels in TBE buffer and the products visualised after staining with ethidium bromide. Positive and negative samples were identified by comparing with the positive controls included with each test kit.

Following species identification, the DNA of some of the samples which tested positive was quantified by repeating the PCR but using the primers from the species kit with a qPCR Master Mix (GoTaq® qPCR Master Mix, Promega, part code A6001). Reactions were run on the Techne PrimeQ real-time thermal cycler[3] using the same protocol as the species identification kit but including fluorescence readings at the end of each extension step. The data was analysed using the Quantification analysis module of Quansoft.

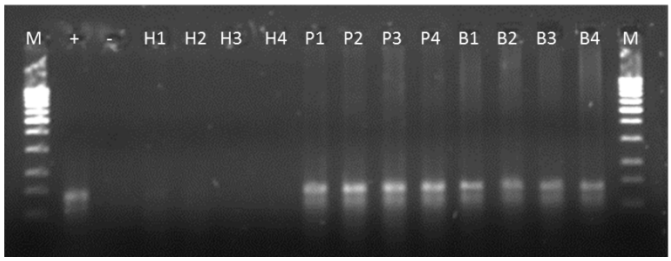
Results

Yields from the DNA extractions of meat samples were greater than those from hair (Table 1) since the samples were more completely digested by overnight incubation with proteinase K. Although horse hair samples did not digest completely, horse hair did amplify positively in the equine species test. The swab samples H3 and H4 did not amplify indicating either the presence of PCR inhibitors or degradation of the DNA (Figure 1).

Equine species test



Porcine species test



Bovine species test

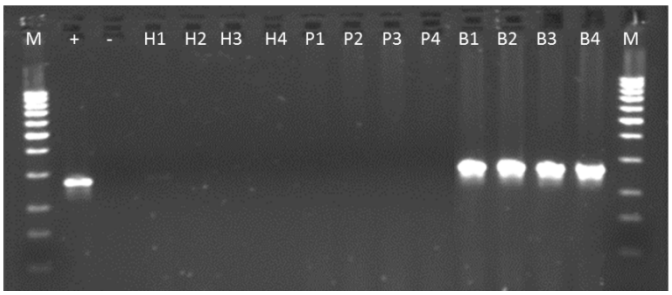


Figure 1: Agarose gels showing PCR products obtained from the three species identification tests.
M = molecular weight markers;
+ = positive control;
- = negative control;
H1-H4 = horse samples;
P1-P4 = porcine samples;
B1-B4 = bovine samples.

Figure 1 shows that all samples gave positive reactions for their species. However the porcine DNA test revealed that the DNA extracted from minced beef also contained some pork contamination.

In order to quantify how much pork contamination was present in the minced beef samples, a quantitative PCR assay was performed using real-time PCR. The pork sample, P1, was serially diluted 1 in 10 to give a series of five standards ranging from 5.9µg/ml to 5.9 x 10^{-4}µg/ml porcine DNA. 2µl of these standards

[2] http://www.techne.com/product.asp?dsl=925
[3] http://www.techne.com/product.asp?dsl=924

were amplified in triplicate reactions using the Techne PrimeQ real-time system to produce a standard curve. 2µl of each of the undiluted minced beef DNA samples were also amplified in triplicate.

Figure 2 shows the amplification curves for the porcine DNA standards (shown in red) and the minced beef samples (shown in green). Samples with a higher initial target concentration begin to amplify at an early cycle number whereas samples with fewer targets amplify much later. The point at which the amplification curve crosses a set threshold is determined and is known as the Cq (quantification cycle) value. The Cq value is plotted against the log of the standard concentration to produce the standard curve.

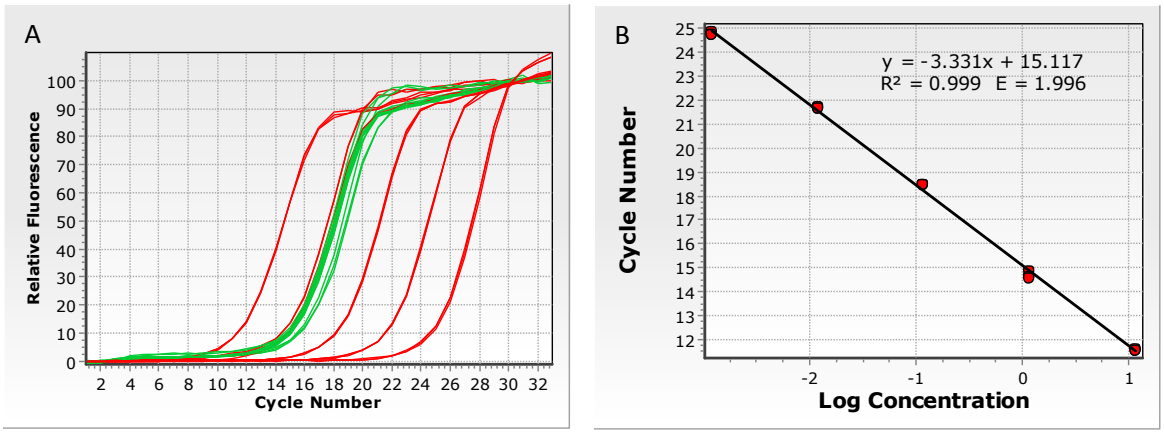


Figure 2: Amplification curves (A) and standard curve (B) for the quantitative porcine DNA assay. Standard porcine DNA replicates are shown in red and the unknowns (minced beef DNA samples) in green. The Cq value for each sample and unknown is determined by the Quansoft software and is used to plot the standard curve.

Using the curve constructed from the porcine DNA standards, the concentration of the unknowns can be calculated. These values are presented in Table 2.

Sample	Pork DNA contamination (ng/µl)	Total DNA concentration (ng/µl)	% Pork DNA contamination
Beef 1	0.338	65.89	0.51%
Beef 2	0.625	69.16	0.90%
Beef 3	0.560	66.26	0.85%
Beef 4	0.635	72.21	0.88%

Table 2: Amounts of porcine DNA detected in the minced beef DNA samples and the relative % contamination levels.

The results indicate that the minced beef sample was contaminated with between 0.5% to 0.9% pork. The origins of the contamination can only be speculated but it is possible the pork contamination was carried over in the processing equipment used for mincing the beef. This may also explain the variation in contamination as this is highly dependent on the homogeneity of the bulk sample.

Conclusions

This study has demonstrated the successful use of the Techne Prime thermal cycler to detect contamination in meat samples using commercial PCR-based species identification kits. In an extension to this, the kit primers were added to a qPCR Master Mix to enable quantification of the contamination using the PrimeQ real-time PCR system.

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