Identification of different meat species by the Agilent Fish ID solution on the Agilent 2100 Bioanalyzer

Application Note

Food Authenticity, Food Testing

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Abstract

In food processing plants, a large number of various ingredients originating from different animal species are used as part of the production process. Sometimes this is done on the same production line. The Agilent Fish ID solution was developed to identify the species of fishes from food samples of different processing levels. This application note investigates its abilities to detect the presence of non-fish species, specifically mammalian or avian DNA originating from dairy products or meat.

Introduction

The Agilent Fish ID solution was introduced to allow fast and easy identification of fish species from raw, cooked, or otherwise processed fish samples. Food manufacturers are using a large number of ingredients in the preparation of their products. This includes dairy products and meat from various mammalian or avian species. These can be part of seafood preparations, either as wanted ingredient or as a contamination from the production process. The aim of this study was to determine the ability of the Fish ID solution to detect mammalian or avian DNA using meat samples.

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Materials and Methods

Meat samples

Beef, pork, wild boar, lamb, chicken, turkey, and duck samples were obtained from local supermarkets or butchers.

Isolation of DNA from meat tissue

DNA was isolated using the protocol described in the Agilent DNA Isolation kit which comes as part of the Fish ID Ensemble. DNA concentrations and 260/280, 260/230 ratios were checked using a Nanodrop ND-2000 spectrophotometer (Thermo Fisher Scientific).

Amplification of *Cyt*b target sequence

For the amplification of the *Cyt*b target sequence, 1 µl of the isolated DNA was used according to the protocol of the Agilent PCR-RFLP Fish ID kit. As a positive control, the kit supplied *Salmo salar* DNA was also amplified as described in the manual. Successful amplification of all samples as well as a clear negative control (NTC) was validated using a DNA 1000 assay on the Agilent 2100 Bioanalyzer.

Restriction digestion of the PCR products

Following the protocol of the Agilent PCR-RFLP Fish ID kit, a 2.5 µL aliquot of the PCR reaction was used in the restriction digestion with each of the kit supplied enzymes *Dde* I, *Hae* III, and *Nla* III.

Analysis of restriction patterns using the Bioanalyzer

The digested samples and the positive control salmon DNA were run on a DNA 1000 chip according to protocol. For each sample, the three independent digests were loaded in consecutive wells, allowing the analysis of four samples per chip. The resulting electropherograms were analyzed using the 2100 Bioanalyzer Expert software.

Results

DNA of sufficient yield and quality was prepared from all tissue samples using the Agilent DNA Isolation kit provided as part of the Fish ID solution. Two independent DNA samples for each of the meat tissues were used in the PCR together with the salmon positive control DNA supplied by the kit and a no template control. The results of the PCR reactions were validated on the 2100 Bioanalyzer using a DNA 1000 assay (Figure 1). For mammalian samples, a single clear PCR product was obtained similar to the salmon positive control. Turkey showed a reproducible double band, whereas chicken and duck samples resulted in a large number of unspecific amplicons present in the reaction.

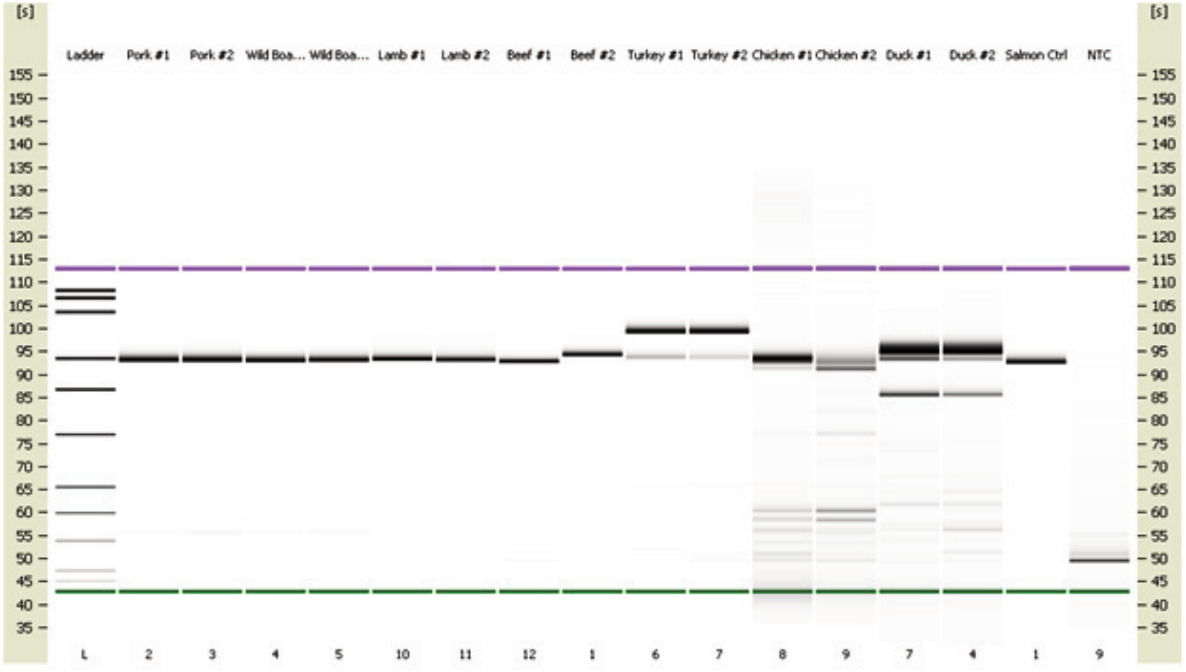

Figure 1: ***Analysis of Cytb PCR results.*** *Two independent meat DNA samples, the salmon (S. salar) positive control and a no-template control were analyzed on a Bioanalyzer using a DNA1000 assay. The virtual gel image shows a comparison file assembled of all samples using the Expert software. Lane 1: Ladder, lane 2 + 3: Pork, lane 4 + 5: Wild boar, lane 6 + 7: Lamb, lane 8 + 9: Beef, lane 10 + 11: Turkey, lane 12 + 13: Chicken, lane 14 + 15: Duck, lane 16: S. salar positive control, lane 17: NTC.*

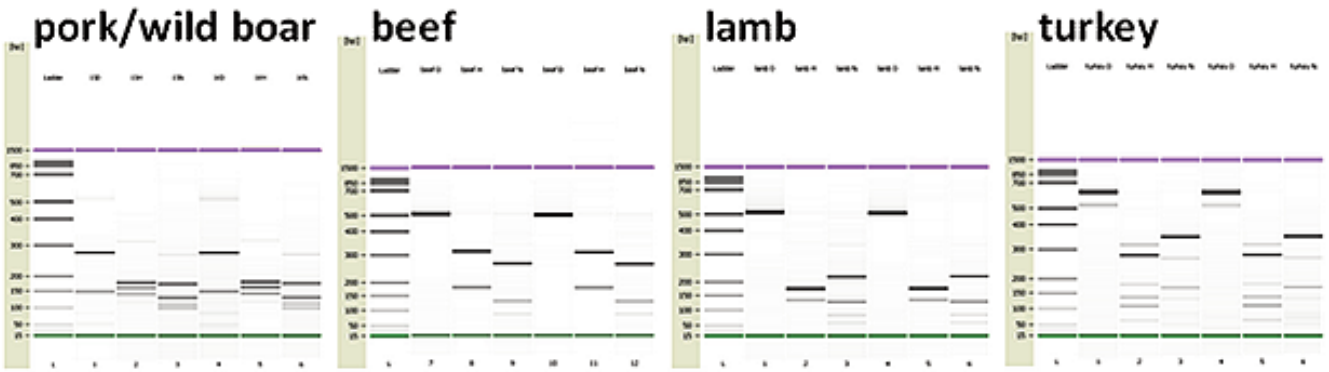

Figure 2. Restriction digestion analysis on the Agilent 2100 Bioanalyzer. The virtual gel images show the pattern combination for the restriction digestion of PCR products obtained from two independent meat tissue samples of pork, beef, lamb and turkey. Each panel shows a lane containing the ladder, Dde I, Hae III, and Nla III digestion pattern for sample 1, followed by sample 2 for the same tissue. Pork and wild boar resulted in the same pattern, therefore only the pork results are shown.

All PCR reactions, with the exception of the NTC, were subsequently used in a restriction digestion according to the manual of the PCR-RFLP Fish ID kit. As expected from the PCR results, both chicken and duck resulted in a multitude of fragments not producing a conclusive pattern (data not shown). In mammalian tissue and turkey, unique and easily identifiable patterns could be obtained (Fig. 2). Pork and wild boar gave rise to the same pattern combination. Fragment sizes are summarized in Table 1.

Table 1. Fragment Sizes and Standard Deviation in Bp for Pork, Wild Boar, Beef, Lamb and Turkey. The 132 Bp Fragment in Brackets was Consistently Present in Nla III Digests of Turkey but was not Detected with Default Settings in the Expert Software as it was Below the Peak Threshold

	***Dde* I**	***Hae* III**	***Nla* III**
Pork	276 ± 0.6, 149 ± 0.6	180 ± 1.3, 162 ± 1.3, 142 ± 1.7	175 ± 1.0, 130 ± 0.5, 109 ± 1.3, 98 ± 0.8
Wild boar	274 ± 1.0, 149 ± 0.8	181 ± 1.5, 162 ± 1.0, 143 ± 2.0	174 ± 0.8, 130 ± 0.8, 111 ± 1.0, 99 ± 0.6
Beef	505 ± 6.0	313 ± 3.1, 180 ± 0.6	267 ± 1.7, 131 ± 0.6, 87 ± 0.5
Lamb	517 ± 4.2	178 ± 1.0, 171 ± 1.0, 135 ± 1.0	220 ± 1.3, 129 ± 1.0, 84 ± 0.8, 58 ± 0.5
Turkey	624 ± 0.7, 525 ± 0.7	319 ± 0.0, 280 ± 1.4, 180 ± 1.4, 137 ± 0.7, 110 ± 0.7, 64 ± 0.7	352 ± 1.4, 271 ± 0.7, 169 ± 0.7, (132 ± 0.7)

Summary

Due to the design of the primers to cover a wide range of fish species, it was expected that non-fish species would be detected. A small scale validation with a few common mammalian and avian meat tissues showed the capability of the kit to successfully identify pork, beef and lamb. Avian *Cyt*b target DNA on the other hand seems to differ substantially from its mammalian or fish homolog. Only turkey gave rise to a simple, easily identifiable pattern, although producing two different PCR products. A full scale meat species identification solution will require a different primer design or even a different target sequence.

For More Information

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