Challenge

Specific detection of Fish DNA from food and feed samples.

Solution

Efficient extraction of nucleic acids and qualitative analysis of Fish DNA using Analytik Jena's qTOWER³ and the innuDETECT Fish Assay.

Convincing Performance of Identification of Fish using qTOWER³

Introduction

Food and agriculture is a growing industry in which quality control is a significant issue. Costumers expect that processed foods are produced under highly regulated conditions and are precisely declared. To ensure this is the task of the manufacturers and government agencies. Depending on the starting material and the parameters of interest, different methods are used. For species identification molecular biological techniques based on real-time PCR have been established.

Analysis of processed foods e. g. determination of the presence of fish within products is based on this method. Qualitative detection is sufficient but high sensitivity and precise signals are compulsory. The first step to achieve trustworthy results is the extraction of nucleic acids. The very fast, easy to handle and efficient innuPREP DNA Mini Kit for nucleic acid extraction can be used for different starting material like fresh fish, processed foods and feed. Downstream analysis were performed implementing the innuDETECT Fish Assay (by Innuscreen GmbH) on Analytik Jena's qTOWER³ real-time thermal cycler. The whole procedure takes approximately 80 min and convinces with very high sensitivity. Comparable results for innuDETECT Fish Assay and Eurofins DNAnimal Screen Fish Assay were reached.

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Materials and Methods

Samples and reagents

- Fresh or smoked fish samples from different species
- innuPREP DNA Mini Kit (845-KS-1041050, Innuscreen GmbH)
- innuDETECT Fish Assay (845-IDF-0100096, Innuscreen GmbH)
- DNAnimal Screen Fish Assay (5422211310, Eurofins)

Instrumentation

- qTOWER³ (e.g. 844-00554-2, including color modules 1 and 2, Analytik Jena GmbH)
- CFX 96 (BioRad)

Procedure

In order to evaluate the performance of the innuDETECT Fish Assay different fresh and smoked fish samples were analyzed. The extraction of nucleic acids from 400 µg staring material was performed according to the instruction of use. Afterwards, qualitative detection of a fish specific target gene (gDNA) was realized with qTOWER³ and CFX 96 as summarized in Table 1a. For comparative analysis with a competitor detection kit the recommended PCR program for DNAnimal Screen Fish Assay was used (Table 1b).

Table 1a: PCR program for qTOWER³ and innuDETECT Fish Assay

Step	Cycle	Profile	Temperature	Holding time
1	1	Initial denaturation	95 °C	120 sec
2	45	Denaturation	95 °C	10 sec
		Annealing/Elongation*	62 °C	45 sec

* Data acquisition: Fluorescence detection (FAM; HEX)

Table 1b: PCR program used for comparative qualitative Analysis (DNAnimal program)

Step	Cycle	Profile	Temperature	Holding time
1	1	Initial denaturation	95 °C	10 min
2	35	Denaturation	95 °C	15 sec
		Annealing/Elongation*	60 °C	90 sec

* Data acquisition: Fluorescence detection (FAM; HEX)

Results and Discussion

In order to get an impression of the specificity of the innuDETECT Fish Assay different sample materials were tested on qTOWER³ and CFX 96 (Table 2). Ten samples of fresh as well as smoked fish were analyzed. All of the 9 species were detected as positive, emphasizing the specificity of the assay. Furthermore, among others (data for additional species are not shown) nucleic acids from human, chicken, beef, and cancer samples were extracted and eluates analyzed applying the innuDETECT Fish Assay. Taking the negative reactions for these species into account, no cross-reactivity was detectable.

Table 2: Amplification of a fish specific target gene

Standard	Detection qTOWER³ (Analytik Jena)	Detection CFX 96 (BioRad)
Pike-perch (*Sander lucioperca*) fresh	+	+
Soused herring (*Clupea harengus*)	+	+
Catfish (*Pylodictis olivaris*) fresh	+	+
Salmon (*Salmo salar*) fresh	+	+
Salmon (*Salmo salar*) smoked	+	+
Gilt-head bream (*Sparus aurata*) fresh	+	+
Carp (*Cyprinus carpio*) fresh	+	+
Salvelinus (*Salvelinus alpinus*) fresh	+	+
Atlantic Mackerel (*Scomber scombrus*) fresh	+	+
Rose fish (*Sebastes norvegicus*) fresh	+	+
Human	-	-
Chicken	-	-
Beef	-	-
Cancer	-	-

Three different samples and one positive control were analyzed to compare the performance of the innuDETECT Fish Assay to the DNAnimal Screen Fish Assay. The qTOWER³ was used for detection. Table 3 summarizes the Ct values which were determined. Using the innuPREP DNA Mini Kit for nucleic acid extraction almost identical results were achieved which shows similar performance of both assays. However, one advantage of the innuDETECT Fish Assay is an optimized PCR protocol with run time of approximately 80 min (DNAnimal Screen Fish Assay approx. 2 h).

Table 3: Performance comparison of innuDETECT Fish Assay and Eurofins DNAnimal Screen Fish Assay both run on Analytik Jena's qTOWER³.

Standard	innuDETECT Fish Assay [Ct value]	DNAnimal Screen Fish Assay [Ct value]
Salmon fresh	24.18	24.51
Gilt-head bream fresh	22.5	22.6
Rose fish fresh	23.53	23.68
Eurofins PK 15 copies/PCR	33.84	33.13

Despite similar Ct values determined with innuDETECT Fish Assay and DNAnimal Screen Fish Assay the amplification curves show differences. Raw data (Figure 1) demonstrate higher background signals for the DNAnimal Screen Fish Assay which could negatively influence set-ups with low concentrated samples. False negative results are possible consequences.

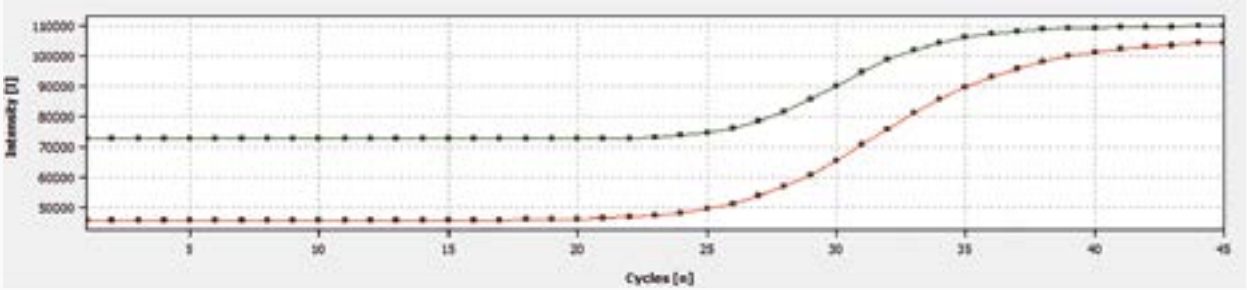

Figure 1: Raw data on qTOWER³; detection using the innuDETECT Fish Assay (red), detection using the DNAnimal Screen Fish Assay (green)

Conclusion

Real-time PCR has been established as a reliable, precise, and fast application for animal species identification. Analytik Jena offers a solution for the detection of fish DNA applying Fish identification Assays on the Analytik Jena's qTOWER³ real-time thermal cyclers. Both the innuDETECT Fish Assay (by Innuscreen GmbH) and the DNAnimal Screen Fish Assay (by Eurofins) can successfully be implemented on qTOWER³ showing the ideal flexibility as well as superior performance of this high-quality real-time thermal cycler family. In case of assays belonging to the innuDETECT product family the whole procedure from nucleic acid extraction to detection of the target DNA takes approximately 80 min.

Headquarters
Analytik Jena GmbH
Konrad-Zuse-Strasse 1
07745 Jena · Germany

Phone +49 3641 77 70
Fax +49 3641 77 9279

info@analytik-jena.com
www.analytik-jena.com

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