Challenge

Reliable detection of porcine DNA in fresh and processed food.

Solution

Implementation of innuDETECT Pork Assay in qTOWER³ for qualitative and quantitative detection of pork DNA.

Precise and Robust Determination of Porcine DNA in Food Samples

Introduction

Analytical molecular methods are currently recognized tools for the determination of DNA in foods. To assure a high level of food and nutrition quality as well as authentication of unintended ingredients such as pork, accurate and highly sensitive animal species identification and the detection of substitutes are two of the main challenges in food industry. However, in order to gain acceptance and confidence in reliability of results obtained by such methods from different laboratories, analytical methods have to satisfy certain quality criteria. Analytik Jena's qTOWER³ real-time thermal cyclers are powerful and highly flexible instruments for the implementation of various real-time PCR assays.
The implementation of the innuDETECT Pork Assay (by IST Innuscreen GmbH) on qTOWER³, the TaqMan®-based innuDETECT Pork Assay (by IST Innuscreen GmbH) enables a highly sensitive analysis of pork species.

Materials and Methods

Samples and Reagents

For reference 0.1% (w/w) pork in beef meat standard (Eurofins), genomic DNA of *Sus scrofa domestica* with a concentration of 100 copies/µl (Eurofins) and an in-house designed plasmid with synthetic pork DNA insert (GeneArt) to be used as positive control were applied using the innuDETECT Pork Assay (845-IDF-0010096, IST Innuscreen GmbH).

Instrumentation

The analysis was carried out on the real-time PCR platform qTOWER^3 from Analytik Jena. Furthermore the CFX96 from Bio-Rad and Step-One Plus from ABI were used in comparison.

Procedure

In order to validate the overall performance and sensitivity the 0.1% (w/w) pork in beef meat standard was extracted using the innuPREP DNA Mini Kit (845-KS-1040250, Innuscreen GmbH). The protocol is based on spin filter nucleic acid extraction in conjunction with a patented chemistry based on a certain combination of chaotropic and non-chaotropic salts (Dual-Chemistry Technology). Further analysis of extracted nucleic acids was performed as described in instructions for use of the innuDETECT Pork Assay (IST Innuscreen GmbH). Target amplification as well as detection was performed by real-time PCR analysis using the FAM channel for target and HEX channel for internal control. The PCR program is summarized in Table 1.

Table 1: PCR program for qTOWER^3

Step	Cycle	Profile	Temperature	Holding time
1	1	Initial denaturation	95 °C	120 sec
2	35	Denaturation	95 °C	10 sec
		Annealing/Elongation *	45 °C	45 sec

* Data acquisition: Fluorescence detection (FAM; HEX)

The **analytical sensitivity** was ascertained by analyzing clarified samples with minimal amounts of DNA. Extracted nucleic acids from 0.05% pork in beef meat, one genome equivalent of genomic DNA of *Sus scrofa domestica* and 10 copies of synthetic plasmid DNA served as templates. At least 30 replicates of each sample type were analyzed and a minimum of 95% should be detected positive.

The **linear range** for the innuDETECT Pork Assay (IST Innuscreen GmbH) was determined by analyzing dilution series of porcine DNA ranging from $3x10^0$ – $3x10^5$ genome equivalents per reaction as well as plasmid DNA ranging from $3x10^0$ – $3x10^7$ copies per reaction.

The **robustness** was tested for the PCR setup. At least 10 samples from 0.1% pork in beef meat were amplified using different PCR instruments, with varying annealing temperatures and with deviations in MasterMix compilation.

Results and Discussion

In order to apply a detection kit for routine analysis different performance parameters have to be ascertained. Comparative analysis was performed for Limit of Detection, amplification efficiency as well as robustness. The application of different food samples was also conducted.

Detection of minimal DNA amounts per sample

In order to determine the analytical sensitivity of the innuDETECT Pork Assay different starting materials with minimal concentrations of porcine DNA per sample (n=30) were analyzed. First results demonstrate that 0.05% (w/w) pork in beef meat can be detected. Positive results for all replicates (30/30) were determined. In addition to that, less than one genome equivalent per reaction could be verified using the innuDETECT Pork Assay. Pork DNA fragments are sufficient for positive test results, as it was shown with amplification of synthetic plasmids. Using 10 copies of porcine DNA per reaction, 96% could be detected positively. All results are summarized in the table below.

Table 2: Results of analysis of samples with defined concentration of porcine DNA

Device	Pork DNA detection	Minimal detected amount of DNA
	Pork in beef meat	
qTOWER^3	30/30	≤ 0.05% (w/w) pork in beef meat
CFX96	30/30	
ABI Step-One Plus	30/30	
	Genomic DNA of *Sus scrofa domestica*	
qTOWER^3	30/30	≤ 1 genome equivalent*
CFX96	30/30	
ABI Step-One Plus	30/30	

* Amplification of mitochondrial gene, i.e. several copies per genome

The detection range of the innuDETECT Pork Assay

The linear range of the detection assay was determined by analyzing porcine DNA ranging from $3x10^0$ – $3x10^5$ genomic equivalents per reaction and synthetic plasmid ranging from $3x10^0$ – $3x10^7$ copies per reaction using the qTOWER^3, the CFX96 and the ABI Step-One Plus. Obtained results, shown in Figure 1, cover a linear range over 7 $\log_{10}$ steps. Based on the Ct value mean, the slope of the regression line was used for determination of the PCR method efficiency. For qTOWER^3 using the positive control plasmid as template an efficiency of 94% could be detected. For qualitative singleplex qPCR methods no criteria of acceptance are defined. By the measure of a quantitative PCR method, the PCR efficiency should range from 90 – 110%. Meanwhile, the innuDETECT Pork Assay reaches an optimal result (Broeders et al., 2014). The calculated linearity R^2 = 0.9955, an additional criterion for method validation ($R^2 \geq 0.98$), emphasizes the assay performance.

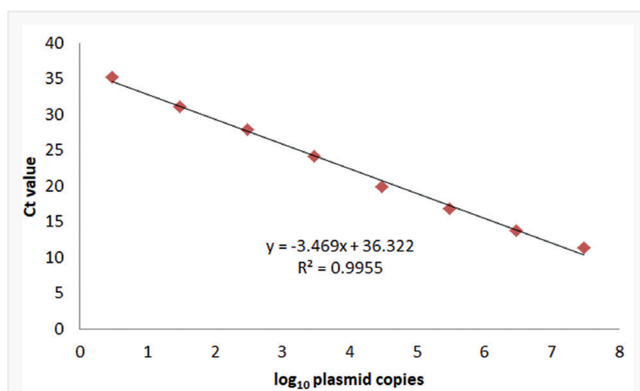

Figure 1: Linearity of the innuDETECT Pork Assay on qTOWER^3 using plasmid DNA. The linear regression coefficient (R^2) is 0.9955.

Requirement of a robust qPCR setup

To evaluate the robustness of the innuDETECT Pork Assay different experimental conditions were changed. Therefore, 0.1% pork in beef meat was analyzed (n=8) using Analytik Jena's qTOWER³ instrument, with different annealing temperatures (+ 2 °C/- 4 °C) as well as with variations in MasterMix compilation (+/- 30%).

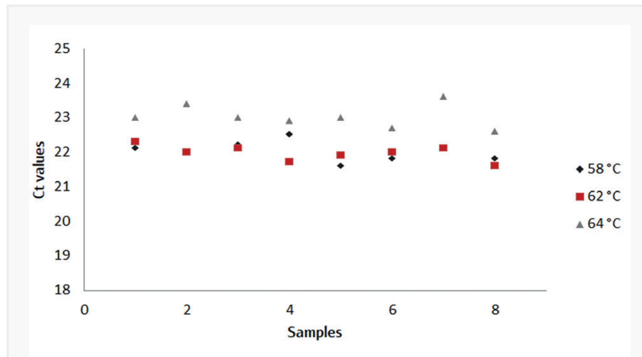

Figure 2: Influence of varying annealing temperature (black: 58 °C, red: 62 °C, grey: 64 °C) on the assay performance, analysis of 0.1 % pork in beef meet (n=8) on qTOWER³ using the innuDETECT Pork Assay

The changed experimental conditions had no influence in positive assay results. Figure 2 demonstrates the effects of different annealing temperatures on DNA detection. Deviation of the annealing temperature and MasterMix compilation for all qPCR instruments has an impact on real-time PCR results less than 5%. Regarding these experimental conditions the performance of the innuDETECT Pork Assay implemented on Analytik Jena's qTOWER³ is convincing.

Conclusion

Real-time PCR has been established as reliable, precise and fast application for animal species identification. Analytik Jena offers a solution for the detection of porcine DNA applying the innuDETECT Pork Assay on qTOWER³. In order to validate the detection system's different performance criteria were analyzed. The robust innuDETECT Pork Assay convinced with a limit of detection ≤ 0.05% (w/w) pork in beef meat. In addition to that, the assay was used for successful detection of porcine DNA in fresh and processed food.

References

Broeders, S.; Huber, I.; Grohmann, L.; Berben, G.; Taverniers, I.; Mazzara, M.; Roosens, N. and Morisset, D.; GUIDELINES FOR VALIDATION OF QUALITATIVE REAL-TIME PCR METHODS. Trends Food Sci Technol. 2014, 37, pages 115-126

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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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