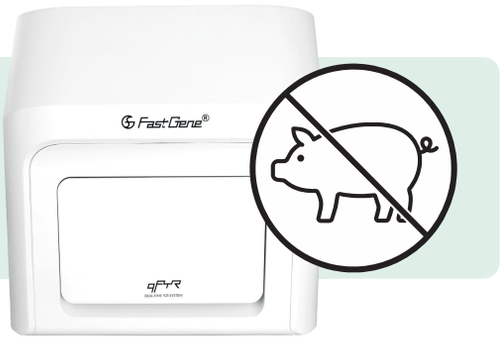
Reliable Detection of Porcine DNA for Food Authentication

RapidFinder™ Pork ID Kit (Thermo Scientific™) on the FastGene® qFYR Real-Time PCR System

Product	FastGene® qFYR Real-Time PCR System (FG-QPTC01, FG-QPTC02)
Distributor	NIPPON Genetics EUROPE GmbH



The following data were provided by NIPPON Genetics EUROPE.

Summary

A ready-to-use pork identification kit was run on the FastGene® qFYR using porcine genomic DNA isolated from muscle tissue. The six-point dilution series showed linear, reproducible amplification, and the kit controls supported clear qualitative interpretation. The results demonstrate practical compatibility of the qFYR with this duplex FAM/VIC food-authentication assay.

Introduction

Reliable species identification is important for food-label compliance, halal and kosher assurance, and consumer confidence. Porcine material may enter a product through incorrect labeling, shared production equipment, insufficient cleaning, or animal-derived ingredients and processing aids. Because even small amounts may be relevant, laboratories require a sensitive and specific method that can be applied in routine workflows.

Real-time PCR detects species-specific DNA and is well suited to this task. Ready-to-use assays simplify reaction setup and provide defined controls for result interpretation. This study evaluated the FastGene® qFYR Real-Time PCR System with the RapidFinder™ Pork ID Kit (Thermo Scientific™). Porcine genomic DNA isolated from muscle tissue was used as a target material. The aim was to show that the qFYR can generate reliable amplification, standard-curve performance, and results with a commercially available pork-detection kit.

Materials and Methods

Instrument and assay.

Reactions were run on the 96-well FastGene® qFYR Real-Time PCR System (FG-QPTC01, NIPPON Genetics EUROPE). The 4+1-channel qFYR (FG-QPTC01) supports the FAM and VIC dyes used by the RapidFinder™ Pork ID Kit (Thermo Scientific™). The kit detects the porcine target in FAM channel and includes an internal positive control (IPC) detected in VIC channel to identify invalid or inhibited reactions.

Test material and controls.

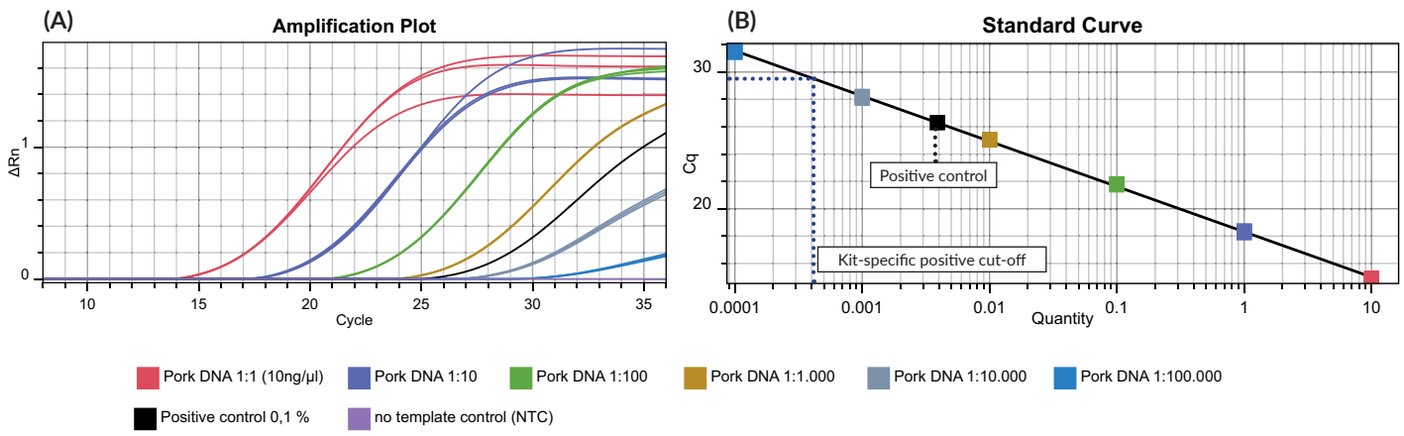
Porcine genomic DNA isolated from muscle tissue (Jena Bioscience) was adjusted to 10 ng/µL. The kit positive control, containing 0.1% porcine DNA in a background of fish DNA, and a no-template control (NTC) were included.

Experimental design.

Six ten-fold standard levels were prepared from the 10 ng/uL working solution (from undiluted to 1:100,000) and tested in triplicates. The positive control and NTC were run on the same plate. Reaction setup and thermal cycling followed the kit instructions. Pork-target amplification was analyzed in FAM channel and the IPC in VIC channel using qFYR Analysis Studio v2.1.2. Cq values from the dilution series were used for linear regression and calculation of amplification efficiency.

Results and Conclusion

Results (Figure 1).
The porcine DNA standards amplified over five orders of magnitude, from a mean Cq of 14.87 at 10 ng/uL to 31.50 at the 1:100,000 dilution. Replicate variation was low at every level (SD 0.021-0.075 Cq). Linear regression gave a slope of -3.312, an R^2 of 0.9996, and an amplification efficiency of 100.4%. A Cq of 26.29 was obtained for the kit positive control, falling within the standard-curve range, whereas no pork-target amplification was observed in the NTC. A preliminary duplex-control check also showed the expected FAM and VIC signals, confirming channel compatibility (data not shown).
For qualitative sample interpretation, the kit manufacturer defines the positive cut-off as the positive-control Cq plus 3.32 cycles. With the measured control Cq of 26.29, the kit-specific positive cut-off was 29.61. A sample with Cq <= 29.61 is therefore interpreted as positive, while a later Cq or no Cq is interpreted as negative, given that the IPC results are valid. The standard curve remained linear beyond the kit-specific qualitative cut-off, with reproducible amplification observed for the 1:100,000 dilution. This demonstrates the strong analytical response of the qFYR, although signals above the cut-off are classified as negative according to the kit instructions.



(C)

Pork DNA Dilution	Quantity [ng/µl]	Cq (mean)	Cq SD
1:1	10	14.87	0.038
1:10	1	18.32	0.071
1:100	0.1	21.79	0.041
1:1,000	0.01	25.05	0.021
Positive control (0.1%)	**0.0039**	**26.29**	**–**
1:10,000	0.001	28.15	0.068
1:100,000	0.0001	31.50	0.075
No template control (NTC)	–	–	–

Figure 1, Porcine DNA detection with the RapidFinder™ Pork ID Kit (Thermo Scientific™) on the FastGene® qFYR (graphs generated with qFYR Analysis Studio Software). (A) Amplification Plot (FAM channel) for the six ten-fold dilutions, kit positive control, and NTC. (B) Standard Curve generated from mean Cq values of triplicate porcine genomic DNA standards; the kit positive control (black) and kit-specific positive cut-off (dashed purple lines) are shown separately. Linear regression: slope -3.312, R^2 = 0.9996, efficiency 100.4%. (C) Mean Cq values and replicate variation. The NTC showed no pork-target amplification.

Conclusion.
The results demonstrate that the FastGene® qFYR Real-Time PCR System is very well suited for pork DNA detection using a commercially available, ready-to-use qPCR kit. The system generated clear amplification, a highly linear standard curve, even beyond the positive cut-off provided by the kit manufacturer, and reliable detection across a broad concentration range. The successful detection of the kit-specific internal control further confirmed robust performance of the assay on the qFYR.

Importantly, the standard was prepared from pork genomic DNA isolated from muscle tissue, providing a relevant sample background for meat and food-testing applications. Together, these results show that the FastGene® qFYR provides the sensitivity, fluorescence detection performance, and reliable qPCR results required for routine food authenticity and contamination testing, making it a strong platform for laboratories using established commercial detection kits.

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