Food adulteration detection using Clarity™ Digital PCR System

I. Overview

The adulteration of meat products with materials from other species with lower cost or greater availability is increasingly observed in recent years. As a result, species identification in meat products is warranted to protect consumers from misleading labeling of meat for religious and/or health reasons[1]. Different analytical methods based on protein or DNA analysis have been applied for food adulteration detection. Among these, polymerase chain reaction (PCR)-based technologies have shown to be effective in distinguishing species in meat products[2]. In this study, we show that digital PCR using the Clarity™ system is a promising approach for detection of minute traces of adulterants in meat samples.

II. Clarity™ digital PCR system detected food adulterant to a level of 0.003%

DNA samples were prepared by mixing isolated *Sus scrofa* (pork) DNA into *Gallus gallus* (chicken) DNA and analyzed using an EvaGreen® dye-based assay. Prepared reaction mixes were evenly distributed into about 10,000 partitions within the Clarity™ tube-strip and then amplified via PCR. As shown in Table 1, as low as 0.003% of pork DNA was detected. In addition, the plot of the obtained data against the expected pork proportion showed a strong linear relationship with an R^2 value of 0.9909 (Figure 1), indicating a high accuracy of the Clarity™ system.

Table 1. Detection of spiked pork DNA using Clarity™ digital PCR system. The EvaGreen® dye-based digital PCR assay was designed to amplify and detect chicken and pork DNA fragments using primer sets listed in Table 2. Pork and chicken DNA were first extracted and diluted to the same concentration. Following which, pork DNA was mixed into chicken DNA to yield the following percentages of pork/chicken DNA: 5%, 1%, 0.1%, 0.05%, 0.01% and 0.001%. Results shown are representative of two independent experiments performed in duplicates with 6 no template controls (NTC).

Expected Percentage (%)	Chicken		Pork		Measured Percentage (%)
	Measured Concentration (copies/µL)	Relative Uncertainty	Measured Concentration (copies/µL)	Relative Uncertainty	
5	59.2	0.03	2.63	0.42	4.443
1	63.34	0.03	0.81	0.41	1.279
0.1	3035.03	0.06	4.6	0.02	0.152
0.05	3694.68	0.01	2.37	0.04	0.064
0.01	3583.44	0.02	0.39	0.45	0.011
0.001	3433.97	0.01	0.12	0.80	0.003
NTC	0	-	0	-	0

©JN Medsys Pte. Ltd.

Figure 1. Plot of measured pork proportion against its expected value.

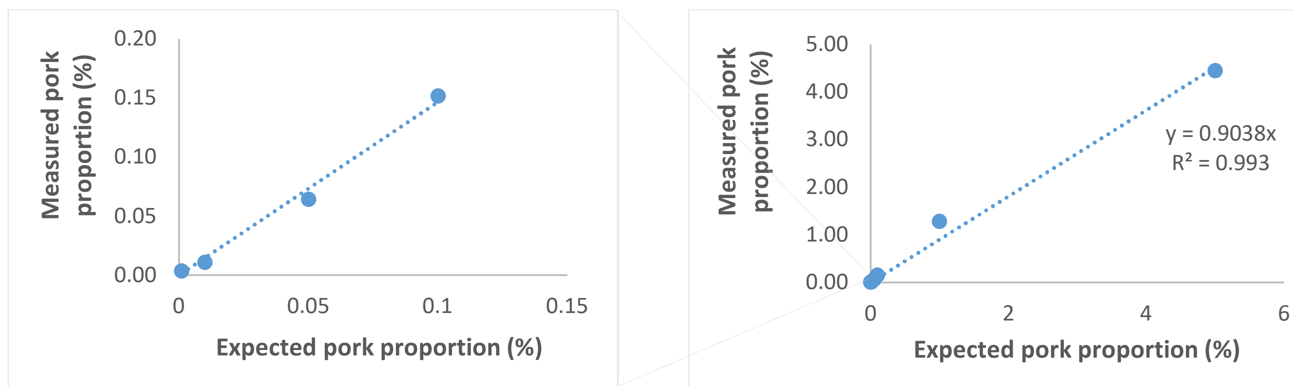

Table 2. List of primers used.

Oligonucleotide	Sequence (5′-3′)
Sus scrofa (pork) Forward primer	TCCTGCCCTGAGGACAAATA
Sus scrofa (pork) Reverse primer	TGATGAGATTCCGGTAGGGT
Gallus gallus (chicken) Forward primer	ATTCTGGGCTTAACTCTCATACT
Gallus gallus (chicken) Reverse primer	GTTCGTTGTTTAGATTTGTGGAG

III. Conclusion

Here we demonstrated that Clarity™ digital PCR system could detect target species at a very low level (0.001%), which was about 100 times more sensitive than qPCR. The accuracy and sensitivity of the Clarity™ system is crucial for detection of adulteration of meat products. Also capable of detecting and identifying foodborne pathogens, Clarity™ is believed to have direct and valuable implications for food safety and consumer health.

References

1. Doosti A, Ghasemi Dehkordi P, Rahimi E (2014) Molecular assay to fraud identification of meat products. J Food Sci Technol. 51:148-52.
2. Ong SB, Zuraini MI, Jurin WG, Cheah YK, Tunung R, Chai LC, Haryani Y, Ghazali FM, Son R (2007) Meat molecular detection: sensitivity of polymerase chain reaction-restriction fragment length polymorphism in species differentiation of meat from animal origin. ASEAN Food J. 14(1):51–59.

Find out more on our website: www.jnmedsys.com
Need technical support? Contact our scientists: techsupport@jnmedsys.com
For Research Use Only. Not for use in diagnostic procedures.