Application of DNA markers for discrimination between Japanese and Australian Wagyu beef

Fuki KAWAGUCHI,[1] Yuto KITAMURA,[1] Ryuji NAKAJIMA,[2] Masayoshi TAKAHASHI,[2] Hiroaki Goto,[2] Yuichi WASHIDA,[3] Yoshikazu YAMAMOTO,[4] and Shinji SASAZAKI,[1] Hideyuki MANNEN,[1,*]

[1] Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural Science, Kobe University, Kobe 657-8501, Japan, [2] DNA Chip Business Promotion Division, Toshiba Corporation, Tokyo 105-8001, Japan, [3] Graduate School of Commerce and Management, Hitotsubashi University, Tokyo 186-8601, Japan, [4] Research Institute of Scientific Criminal Investigation, Hyogo Prefectural Police, Kobe 650-8510, Japan

*Correspondence: Hideyuki Mannen, Graduate School of Agricultural Science, Kobe University, Nada, Kobe 657-8501, Japan. (E-mail: mannen@kobe-u.ac.jp)

TEL: +81-78-803-5803, FAX: +81-78-803-5803

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ABSTRACT

The objective of this study was to discriminate between original Japanese and Australian Wagyu beef, which is sold in the Singapore markets, using previously developed six DNA markers. To effectively evaluate the six markers for breed identification, the probability of identification as Australian Wagyu beef was calculated based on the estimated allele frequencies using 130 Australian Wagyu individuals. The combined use of six markers would allow the discrimination of Australian Wagyu beef with an estimated probability of 0.776. The probability to discriminate Australian Wagyu from Japanese Wagyu beef was sufficiently high. In addition, Australian Wagyu has maternal mtDNA of *Bos indicus* cattle with moderate high frequency of 0.377. The DNA marker system could also be used as a deterrent force against false sales, and contribute to the reduction and prevention of incorrect or falsified labeling of beef.

Key words*: Australian Wagyu, DNA marker, deterrent force, Japanese Black cattle, misbranded beef*

INTRODUCTION

In Japan, the main beef cattle is Japanese Black cattle, which is generally known as Japanese Wagyu worldwide. The genetic resource was exported to the United States (US) in the 1990's and is now being used to produce Wagyu beef in several countries. Wagyu generally refers to the four Japanese native breeds bred in Japan; however, nowadays the famous brand name Wagyu includes not only Japanese native cattle produced in Japan but also animals or even crossbred Japanese native cattle produced in foreign countries, such as Australia or the US. Both original Japanese and other Wagyu beef are now exported to developed countries as high-class beef. Therefore, Wagyu beef has been competitively sold in several countries, such as Singapore, Shanghai, the US and European countries.

In the beef market, a system for discrimination between Japanese and other Wagyu beef based on molecular techniques is required to detect misbranded beef and guarantee original Japanese Wagyu beef. In our previous study, we developed DNA markers to discriminate between Japanese domestic beef and the US and Australian beef (Sasazaki *et al*. 2007, 2011; Suekawa *et al*. 2010).

This marker system may have the potential to discriminate between original Japanese and other Wagyu beef. This study aimed to apply this DNA marker system to Australian Wagyu beef sold in Singapore to discriminate from

original Japanese beef.

MATERIALS AND METHODS

Australian Wagyu beef samples in Singapore

We purchased 142 commercial Australian Wagyu beef in 13 Singapore markets from July 21st, 2013 to November 4th, 2013. Genomic DNA was extracted from the beef samples according to the standard phenol and chloroform method. To exclude samples from the same individual, we genotyped 47 SNP markers using a DigiTag2 assay to identify the same individuals (Yonesaka *et al*. 2016). After the exclusion, 130 different individuals were determined and used in the present study.

DNA markers for discrimination

We applied six discrimination markers for Japanese domestic and imported beef from the US and Australia (BISNP7, BISNP21, BISNP39, BISNP40, BISNP43 and ND5), developed in our previous studies (Sasazaki *et al*. 2007, 2011; Suekawa *et al*. 2010). Genotyping was performed according to the methods used in the previous studies.

RESULTS & DISCUSSION

We applied six DNA markers developed in our previous studies (Sasazaki *et al*. 2007, 2011; Suekawa *et al*. 2010) to discriminate between Japanese and

Australian Wagyu beef sold in Singapore. Table 1 shows the genotype and allele frequencies in Australian Wagyu beef using the six markers. In all markers, we defined that allele 1 has not been detected in Japanese domestic cattle and allele 2 is fixed in Japanese domestic cattle (Sasazaki *et al*. 2007, 2011; Suekawa *et al*. 2010). The ND5 gene is located on the mitochondrial genome, and allele 1 and 2 originate from *Bos indicus* and *Bos taurus* of cattle, respectively (Komatsu *et al*. 2004; Sasazaki *et al*. 2007). To effectively evaluate the six markers, the probability of identification as Australian Wagyu beef was calculated based on the estimated allele frequency of each marker (Suekawa *et al*. 2010).

[Table 1]

In all markers, allele 1 was observed in Australian Wagyu beef with moderate allelic frequencies (0.081 - 0.377). The combined use of six markers would allow the discrimination of Australian Wagyu beef with an estimated probability of 0.776 (Table 1). This probability is lower than that of normal Australian beef (0.925) calculated by the data of same six markers reported in the previous studies (Sasazaki et al. 2007, 2011; Suekawa *et al*. 2010). However, the probability is sufficiently high to discriminate between Australian Wagyu and Japanese Wagyu beef. Interestingly, Australian Wagyu has maternal mtDNA of *Bos indicus* cattle with a high frequency of 0.377. This molecular result suggests that the Australian Wagyu beef sold in Singapore has

substantial genetic influence from local Australian beef cattle.

In conclusion, a system of six markers exhibited a powerful identification probability of 77.6% for Australian Wagyu from original Japanese Wagyu beef. This marker system could verify and guarantee Japanese Wagyu brand produced in Japan. In addition to these six markers, several markers have been developed for discrimination between Japanese domestic and imported from US and AUS beef in the previous studies (Sasazaki *et al*. 2007, 2011; Suekawa *et al*. 2010). Utilization of these additional markers could provide more accurate and reliable system with higher probability. This DNA marker system could also be useful as a deterrent force against false sales and contribute to the reduction and prevention of incorrect or falsified labeling of beef.

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REFERENCES

Komatsu M, Yasuda Y, Matias JM, Niibayashi T, Abe-Nishimura A, Kojima T, *et al.* (2004). Mitochondrial DNA polymorphisms of D-loop and three coding regions (ND2, ND4, ND5) in three Philippine native cattle: indicus and taurus maternal lineages. *Nihon Chikusan Gakkaiho* **75,** 363–378.

Sasazaki S, Hosokawa D, Ishihara R, Aihara H, Oyama K, Mannen H. 2011. Development of discrimination markers between Japanese domestic and imported beef. *Animal Science Journal* **82,** 67-72.

Sasazaki S, Mutoh H, Tsurifune K, Mannen H. 2007. Development of DNA markers for discrimination between domestic and imported beef. *Meat Science* **77,** 161-166.

Suekawa Y, Aihara H, Araki M, Hosokawa D, Mannen H, Sasazaki S. 2010. Development of breed identification markers based on a bovine 50K SNP array. *Meat Science* **85,** 285-288.

Yonesaka R, Sasazaki S, Yasue H, Niwata S, Inayoshi Y, Mukai F, *et al.* 2016. Genetic structure and relationship of 16 Asian and European cattle populations using DigiTag2 assay. *Animal Science Journal* **87,** 190-196.

Table 1 Genotype and allele frequencies of six discrimination DNA markers

marker	Genotype frequency			Allele frequency		PI[1]
	11	12	22	1	2	
BISNP7	1	27	102	0.112	0.888	0.211
BISNP21	0	28	102	0.108	0.892	0.204
BISNP39	1	25	104	0.104	0.896	0.197
BISNP40	0	21	109	0.081	0.919	0.155
BISNP43	0	21	109	0.081	0.919	0.155
ND5	49	—	81	0.377	0.623	0.377
Total[2]						0.776

[1] Probability of identification as Australian Wagyu beef
[2] Probability of identification combined use of six markers