GENETIC IDENTIFICATION OF TEN CATTLE BREEDS IN THAILAND USING DNA MARKERS

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Abstract

The objective of this work was to develop a DNA markers for identification of ten cattle breeds in Thailand: North Eastern Thai Native cattle (ENC) , Northern Thai Native cattle (WL) , Brahman cattle (BRA), Holstein Friesian cattle (HF); Tak cattle (TK), Thai Milking Zebu (TMZ), KamphaengSaen cattle (KPS), crossbred Native x Brahman (BN), Pon Yang Kham cattle (PYK), and Tajima cattle (TJ), using SNPs locate in Thyroglobulin (*TG*), Melanocortin 1 receptor (*MC1R*), 16S ribosomal RNA (*16SrRNA*) and microsatellite marker locate in Growth hormone receptor (*GHR*). This preliminary study showed that the DNA marker combinations can be successfully used as a part of an effective traceability system for the identification of eight cattle breeds, except KPS and PYK cattle and can be used as a foundation for further development of DNA markers in cattle breed identification.

Key Words –genetic traceability, single nucleotide polymorphism, Thai cattle breeds,

I. INTRODUCTION

Thailand has a total number of beef cattle population has 4.3 million heads, including Thai native cattle /crossbred native cattle about 2.7 million heads (64.13%), synthesis breed and crossbred cattle has 1.3 million heads (32.18%) and fattening beef cattle around 0.15 million heads (3.69%) (DLD, 2014). Thai native cattle breed is known as zebu cattle (*Bos indicus*), like Indian cattle.

Thai native cattle are classified into four breeds (Kow-Lamphun, Kho-Esarn, Kho-Lan and Kho-Chon cattle) showed in different region of Thailand. They are small body size, heat tolerant, insect and disease resistant, good grazers and great reproductive performance, however, the native cattle breed raised in the north-eastern part of the Thailand (Kho-Esarn), also used for beef production, through a crossbreeding system, which cross with exotic breeds as two-way crossbred (Thai native x Brahman, Angus or Holstein Friesian), and three-way crossbred or synthetic line for improve performance and produced the high-quality meat. However, Thai consumers are willing to pay the fattening beef or crossbred beef meat rather than native beef cattle, even though the price of their meat is higher. Hence, the evaluations of genetic identification of cattle breeds are raise in Thailand that purpose for produced high quality meat is necessary. For protect consumers from fraudulent quality claims. There is becoming more challenging, which can be used for breeding strategies and further traceability system for beef cattle breeds in Thailand.

Molecular methods have been widely used for genetic variation, genetic identification and genetic evolution of livestock animal (Visscher et al., 2002), by DNA markers have been shown unique sequences are single nucleotide polymorphisms (SNPs), Microsatellite and Amplified fragment length polymorphism (AFLP) (Dalvit et al., 2007). Thus, in order to develop DNA based breed traceability and authentication protocols, the first step is the identification of breed specific markers with high discriminatory power among breeds, the presence of private alleles or genotype combinations. The aim of this study was to investigate the DNA markers that specific in some cattle breeds in Thailand.

II. MATERIALS AND METHODS

The ten cattle breeds were derived from four purebred cattle: North Eastern Thai Native cattle (Esan) (ENC, N=40), Northern Thai Native cattle (White Lamphun) (WL, N=30), Brahman cattle (BRA, N=40), Holstein Friesian cattle (HF, N=30), and six crossbreed cattle: Tak cattle = 62.5% Charolais: 37.5% Brahman (TK, N=25), Thai Milking Zebu = 75% Holstein Friesian 25% native (TMZ, N=25), KamphaengSaen cattle = 25% native: 25% Brahman: 50% Charolais (KPS, N=10), crossbred Native x Brahman (BN, N=25), Pon Yang Kham cattle = 25% native: 25% Brahman: 50% exotic breed (Charolais or Simmental) (PYK, N=30), and Tajima cattle (TJ, N=25). The samples of BRA, BN, TK, TMZ and TJ cattle blood samples were obtained from the Department of Livestock Development. Esan cattle were obtained from Department of Animal Science, Faculty of Agriculture, Khon Kaen University. HF semen samples and KPS and PYK cattle meat samples were obtained from Chokchai farm. The WL cattle blood samples were obtained from Department of Animal Science, Faculty of Agriculture, Chang Mai University (with courtesy from Assoc. Prof. Dr. Supamit Mekchay)

Extraction of DNA was performed to the samples from different source: 30 µl of white blood cells (ENC, WL, BR, T, TMZ, BRA and TJ) , 30 µl of semen (HF) and 30 mg of meat tissue (PYK and KPS), using a guanidine hydrochloride protocol modified from Goodwin et al. (2007). Afterwards, to determine the concentration of DNA, NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) was used.

To identify genetic variations of ten cattle breeds in Thailand, we screened 23 markers. Among them, we chose 5 markers (*TG*, *16SrRNA*, *GHR*, *MC1R*-1 and *MC1R*-2) based on polymorphism test among the cattle breeds. The polymorphism of *TG* and *MC1R-1* markers were detected by PCR-RFLP methods (Pham et al., 2011; Crepaldi et al., 2003) and including, the new SNPs in *16SrRNA* gene. The variation of *MC1R-2* was detected by High resolution melting, HRM and DNA sequencing. The *GHR* (microsatellite marker) was detected by PCR methods (Lucy et al., 1998).

In this experiment, we used a deterministic approach to look for markers combinations of different genotype fixed within different breeds. The development of simple analysis protocols was made possible without statistical inference (Dalvit et al., 2007).

III. RESULTS AND DISCUSSION

At present, in both microsatellites (STRs) and SNPs are the markers most commonly used identification of animal breed, due to microsatellite are exhibit a high degree of polymorphism (Dalvit et al., 2007), while SNPs have genetic stability, lower rates of genotyping error (Karniol et al., 2009).

The SNP occurs in the 5´ promote region of the *TG* gene and is widely used in marker assisted selection (MAS) programs to improve meat quality in beef cattle. The T allele gives two bands of 473 bp and 75 bp, allele C gives three bands of 295 bp, 178 bp and 75 bp.

The presence of the T296C substitution in the *MC1R*-1 gene created restriction site with the production of three short fragments of 8, 33 and 97 bp; the absence of mutation produces two fragments of 8 and 130 bp that reported by (Crepaldi et al., 2003). This SNP has been observed only in the Italian Holstein breed, similar, we also observed that the TT genotype showed in Holstein Friesian and small number cattle in TMZ.

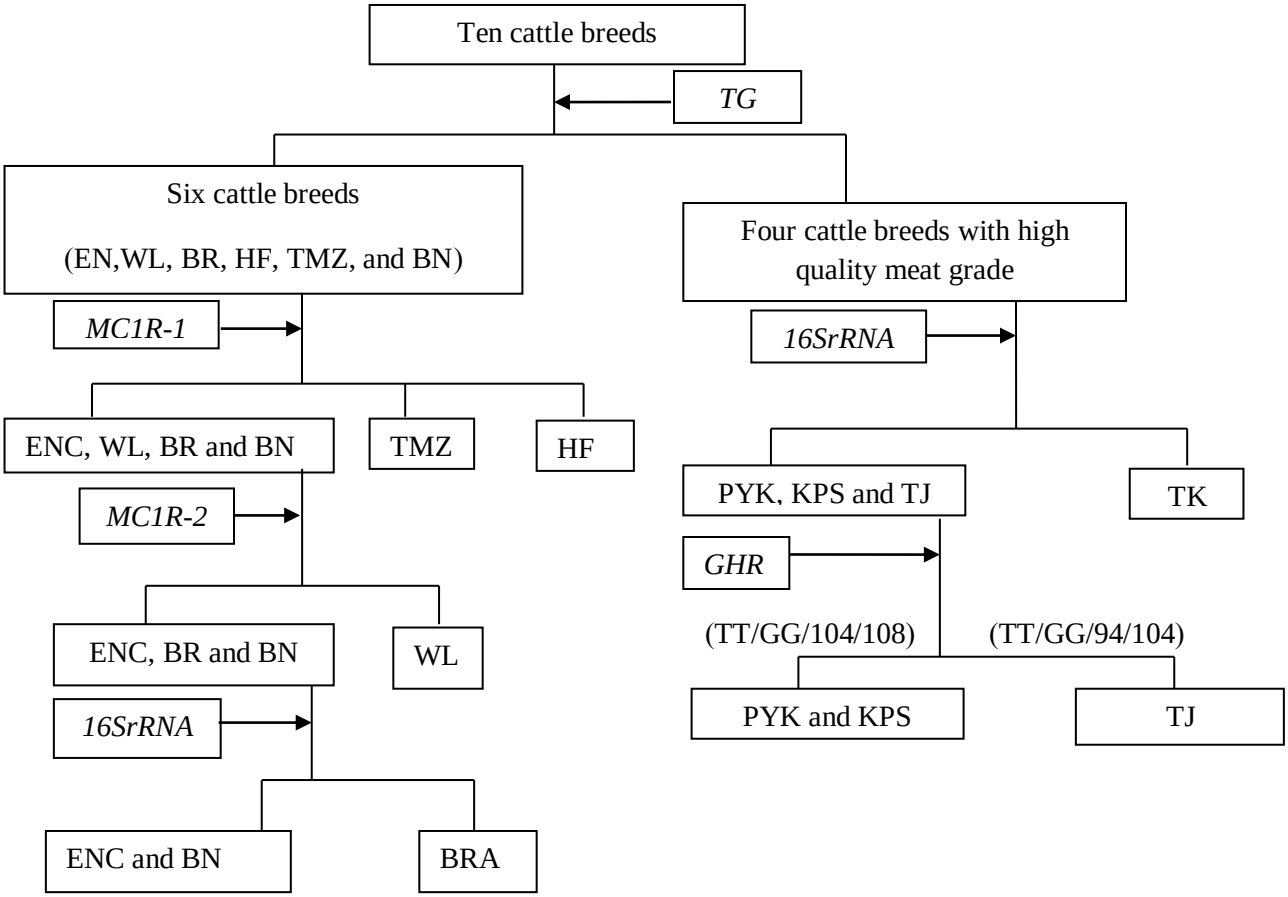
In addition, Mekchay (2008) reported that the four polymorphic sites were found in *MC1R* gene of the White Lamphun cattle at position 296, 416, 663 and 725 bp of open reading frame. Likewise, in our study, the results between the WL cattle and other cattle breeds showed difference. As a result, we have found SNP at position 416 bp change nucleotide C416T of *MC1R-2* markers.

The PCR product of the *16SrRNA* gene were digested with *Apo*I enzyme, two digestion patterns has uncut (512 bp) and cut (335 and 177 bp).

Table 1 Number of animals with genotype of genes

Markers	Genotype	ENC	WL	BRA	HF	TK	TMZ	KPS	BN	TJ	PYK
	count	40	30	40	30	30	25	10	25	25	30
TG	TT	0	0	0	0	3	0	7	0	9	10
	CT	0	0	0	0	11	0	3	0	16	13
	CC	40	30	40	30	16	25	0	25	0	7
MC1R-1	TT	0	0	0	30	0	2	0	0	0	0
	CT	0	0	0	0	0	23	0	0	0	0
	CC	40	30	40	0	25	0	10	25	25	30
MC1R-2	CC	40	0	40	25	25	25	10	25	25	30
	CT	0	2	0	0	0	0	0	0	0	0
	TT	0	28	0	0	0	0	0	0	0	0
16SrRNA	GG	40	40	0	0	21	20	10	25	25	30
	AA	0	0	40	30	9	5	0	0	0	0
GHR	94/94	6	0	40	0	0	0	0	0	0	0
	94/104	34	30	0	0	27	25	0	0	25	0
	104/108	0	0	0	10	3	0	0	25	0	30
	108/112	0	0	0	20	0	0	10	0	0	0

Figure 1. Diagram chart for cattle breeds identification by genotype combinations



Sequence analysis showed that SNP at positions G 177A. However, we found that the GG genotype in Holstein Friesian, Brahman, Tak and other breed showed AA genotype. According to, Yoon et al. (2008) used the mitochondria marker with *SRY* gene could be discriminated between imported beef (*Bos indicus*) and Honwoo cattle *(Bos taurus)*.

A polymorphic $(GT)_n$ microsatellite was identified within the somatotropin receptor gene promoter (growth hormone receptor), generated allele size were the 94-bp allele, 104-bp allele, 106-bp allele, 108-bp allele, and 112-bp allele (Lucy et al., 1998). In our study, using the same microsatellite locus was found the variation of *GHR* gene in cattle breed, 94-bp allele, 104-bp allele, 108-bp allele, and 112-bp allele. The genotype counts in each of cattle breeds are presented in in Table 1 and the genotype combinations were determined from the ten cattle breeds in Fig. 1.

IV. CONCLUSION

Base on this study, purebred cattle (ENC, WL, HF, and BRA) breed is genotypically distinguishable from crossbreed cattle with high quality meat (TK, KPS, TJ and PYK) by use of *TG* genotyping. The use of *MC1R* genotyping approach seems appropriate identity between HF, TMZ and WL. The *16SrRNA* could be discriminated among BRA and BN. However, it could not identify between ENC and BN. For the genetic differentiation within high quality beef breed, we found the *16SrRNA* could be discriminated TK from KPS, PYK and TJ and also, found the *GHR* could be discriminated between 50% Charolais within breed (KPS and PYK) and TJ cattle. The principal polymorphisms at five loci could be useful for meat or milk traceability of purebred cattle and high quality beef breeds. The possibility of establishing a defined genotype for the cattle breeds allows setting an accurate management of breeding programs for genetic improvement and breeding genetic identity preservation over time.

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