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งานวิจัย

Genotyping of Single Nucleotide Polymorphism (SNP) in the Thyroglobulin (*TG*) Gene of Angus x Thai Native Crossbred Cattle

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Abstract

The objective of this study was to identify the single nucleotide polymorphism (SNP) in the thyroglobulin (*TG*) gene of Angus x Thai native crossbred cattle. The SNP reported in *TG* gene which encodes the glycoprotein precursor to the thyroid hormones lies in 5' untranslated region (5'UTR) of the *TG* 5 gene, and has been associated with differences in marbling score in beef cattle. Genomic DNA samples from 73 Angus x Thai native crossbred cattle were amplified the *TG* 5 gene prior to make directly nucleotide sequence of the PCR products. The result indicated that two point mutations in the *TG* 5 gene at position 422 and 552 of the *TG* 5 gene (GenBank accession number X05380) was a C/T polymorphism. Three genotypes of the *TG* 5 gene were classified according to homozygous CC or TT and heterozygous CT of of the nucleotide C or T at the two SNP markers. The genotypic frequencies of the SNP1 marker were 58.9%, 39.7% and 1.4% with genotypes CC, CT and TT respectively, whereas the genotypic frequencies of the SNP2 marker were 0, 68.5 and 31.5 %. The SNP1 marker of *TG* 5 gene had been evaluated for association with marbling, one of carcass composition traits in various beef cattle. A higher percentage of those with more favorable genotype for marbling, known as the 'TT' genotype, achieve a higher marbling grade. Thus, we analyzed *TG* 5 marker of 41 heifers obtained from Thai native cows which were artificially inseminated with 3 Angus semen. High frequency (63.6 - 80.0%) of the genotype CT in 26 heifers obtained from 2 sires with the genotype CT, as well as low frequency (6.7%) of the genotype TT in one of 15 heifers obtained from the sire with the genotype CT. Moreover, low frequency (6.7%) of 15 heifers with the genotype CT obtained from one sire with the genotype CC. The result indicated that the *TG* 5 marker would be a candidate molecular marker for assisting selection of Angus sire to enhance possibility of marbling in Angus x Thai native crossbred cattle. Further study on evaluation of the association of this marker with marbling score need to be taken in Angus x Thai native crossbred cattle. The *TG* 5 marker will be one of several SNP markers for marbling and be used for marker-assisted selection (MAS) in the crossbred beef breeding program.

Keywords : Angus x Thai native, Crossbred cattle, Genotype, SNP, Thyroglobulin

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Introduction

In mammals, an adequate supply of thyroid hormones is not only essential for normal growth and neurological development but also plays an important role in regulating metabolism and can affect homeostasis of fat depots. The biosynthesis of thyroid hormones involves an iodinated precursor protein, thyroglobulin, which may be considered an extreme example of a pro-hormone. Thyroglobulin is a dimeric glycoprotein of relative molecular mass (Mr) 660,000 (660K), which is secreted by the thyrocyte and stored in the lumen of the thyroid follicle. The hormonogenic reaction is extracellular, and involves iodination of tyrosyl residues of thyroglobulin and the intramolecular coupling of a subset of these into thyroxine (T4) and triiodothyronine (T3), which remain part of the polypeptide chain.

The complete primary structure of bovine thyroglobulin, derived from the sequence of its 8,431-base-pair complementary DNA. The 2,769-amino-acid sequence is characterized by a pattern of imperfect repeats derived from three cysteine-rich motifs. Four hormonogenic tyrosines have been precisely localized near the amino and carboxyl ends of the protein (Mercken *et al*., 1985). The bovine thyroglobulin (TG) gene had been investigated by a combination of Southern genomic blotting and direct analysis of cloned gene fragments isolated from a chromosomal DNA library. The entire locus is spread over more than 200,000 base pairs which makes it one of the largest eukaryotic genes studies to date. The coding information is scattered into at least 42 exons, 34 of which have been precisely identified. The bovine TG gene and of its 5' flanking region or 5' untranslated region (5' UTR) showed a significant homology exists between bovine and human thyroglobulin sequences, except for the presence within the ruminant promoter region of a 220-base-pair sequence belonging to the bovine monomer repeated family (de Martynoff *et al*., 1987). The TG 5' flanking region is a consensus sequence for the RNA polymerase III binding site. Thus, variation in this segment had been proposed to account for some of the genetic variation in producing the precursor for thyroid hormones. The variation in the 5'-flanking segment of the TG gene (TG 5) is single nucleotide polymorphism (SNP). The SNPs located at the position 422 and 552 of the TG 5 gene (GenBank accession number X05380) were investigated and had been associated with marbling. Three types of alleles were classified according to single nucleotide mutation (C/T) at these positions (Barendse, 1997). The TG 5 gene test for a marbling quantitative trait loci had been evaluated in long-fed (>250 days on feed) and short-fed (<250 days on feed) in beef cattle. Cattle having allele '3' or TT genotype which the SNPs located at the position 422 and 552 is T and T, are prone to have higher marbling scores than cattle with the allele '2' or CT genotype (Barendse, 1997; Barendse *et al*., 2004).

The objective of this study was to identify SNPs in 5' UTR of the TG 5 gene of Angus x Thai native crossbred cattle.

Materials and methods

Animals

Seventy-three of Angus x Thai native crossbred cattle including 23 steers and 50 heifers obtained from Thai native cows artificially inseminated

to 3 Angus sires with frozen semen under the Thai-Black, high quality beef production project. The project was implemented by Bureau of Biotechnology in Livestock Production, Department of Livestock Development since 2006.

DNA samples

A 10 ml sample of blood were collected from the jugular vein and transferred into EDTA tubes, then submitted to the laboratory within 24 hr. Two hundred ml of the blood samples were extracted DNA by using FlexiGene DNA Kit (QAIGEN, USA) according to the manufacturer's protocol. The DNA samples were kept at -30°C until used.

Amplification of TG 5 gene

The DNA fragment of TG 5 gene was amplified, using the primers TG5U2: 5'- ggg gat gac tac gag tat gac tg-3' and TG5D1: 5'-gtg aaa atc ttg tgg agg ctg ta-3' designed according to the TG5 gene of GenBank accession number X05380 (Barendse *et al*., 2004) as shown in Fig 1. In 50 µl of polymerase chain reaction mixture containing 200 µM of each dNTPs, 1.5 mM of Mg^{2+}, 10 µM of each primers, 0.25 U of Taq DNA polymerase (Platinum Taq DNA polymerase, Invitrogen, USA). Two µl of DNA templates from each sample were used to amplified TG 5 gene using thermal cycler (GeneAmp® PCR System 9700, AB, USA) with denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 1 min, an annealing temperature of 58°C for 30 sec and extension of 72°C for 1 min, then final extension at 72°C for 8 min. The DNA fragment (537 bp) of TG 5 gene were detected on 1.5% agarose gel after staining with ethidium bromide through UV transillumination as shown in Figure 2.

DNA sequencing

The specific DNA fragment (537 bp) of the TG 5 gene of each sample on agarose gel were excised and purified with PCR Clean-Up Gel Extraction Kit (NucleoSpin® Extract II, MN, USA) according to the manufacturer's protocol. Ten ng of purified PCR products were used as a template for DNA sequencing using 3130 Genetic Analyzer (AB, USA) according to the manufacturer's protocol.

Allelic type and Genotyping

A SNP occurs when corresponding sequences of DNA from different individuals differ at one DNA base, for example where the sequence of TG 5 gene GATCCCC changes to GATTCCC. This contains two alleles of C and T. SNPs typically have three genotypes, would be CC, CT and TT. Two SNPs occurred in the TG 5 gene at the position 422 and 552 of the TG 5 gene (GenBank accession number X05380) as shown in Figure 1. Allelic, genotyping and haplotyping were designated by several authors. For example, three haplotypes were classified according to single nucleotide mutation (C/T) at these positions. Allele '1' (CC) of the TG 5 gene had both nucleotide C at SNP1 and SNP2. Allele '2' (CT) had a nucleotide C and T, whereas allele '3' (TT) had both nucleotide T at SNP1 and SNP2. Then, genotyping were designated as g11, g12, g13, g22, g23, g33 (Barendse *et al*., 2004). The commercially available GeneSTAR for marbling test measures the specific thyroglobulin gene polymorphism and identifies cattle as having 0, 1, or 2 copies of the T allele; these are identified as 0-STAR, 1-STAR, or 2-STAR, which corresponded

to genotype of g22, g23, or g33, respectively. On the other hand, the 0-STAR, 1-STAR, or 2-STAR corresponded to homozygous or heterozygous of the nucleotide CC, CT and TT at the SNP1 position.

Results

The PCR products of the TG 5 gene

DNA fragments (537 bp) of the TG 5 gene of Angus x native crossbred cattle were amplified by PCR prior to DNA sequencing as shown in Figure 1.

Nucleotide sequence of the TG 5 gene

Nucleotide sequence at 5'-flanking region or 5'-untranslated region (5' UTR) of the TG 5 gene (GeneBank accession number X05380) was identified two mutations in 73 DNA samples from Angus x Thai native crossbred cattle. The point mutation was designed to SNP1 and SNP2 at position 422 and 552 as shown in Figure 2.

In Figure 3, single nucleotide polymorphism T/C were detected by DNA sequencing machine at the two SNPs of the TG 5 gene with heterozygous T/C or homozygous C or T. It was noticed that the peaks of heterozygous T/C was lower than the homozygous C or T.

SNP Genotype frequencies

In Table 1, SNP genotype frequencies of two SNP markers of the TG 5 gene in 73 Angus x Thai native crossbred cattle were demonstrated. The genotypic frequencies of the SNP1 marker were 58.9, 39.7 and 1.4 % with genotypes CC, CT and TT respectively, whereas the genotypic frequencies of the SNP2 marker were 0, 68.5 and 31.5 %.

The SNP1 marker of the TG 5 gene had been evaluated for association with marbling, one of carcass composition traits in various beef cattle. The favorable T allele of TG 5 gene has been associated with increases in marbling score in Angus cattle. Thus, we analyzed the SNP1 marker in the TG 5 gene of 41 heifers obtained from Thai native cows which were artificially inseminated with 3 Angus semen.

Table 1 Genotypic frequencies for the two SNP markers of *TG* 5 gene in Angus x Thai native crossbred cattle

SNP markers	No. of animals	SNP genotypes of *TG* 5		
		CC	CT	TT
SNP1	73	43	29	1
		(58.9%)	(39.7%)	(1.4%)
SNP2	73	0	50	23
		(0%)	(68.5%)	(31.5%)

In Table 2, high frequency (63.6-80.0%) of the genotype CT in 26 heifers obtained from 2 sires with the genotype CT, as well as low frequency (6.7%) of the genotype TT in one of 15 heifers obtained from a sire with the genotype CT. Moreover, low frequency (6.7%) of 15 heifers with the genotype CT obtained from one sire with the genotype CC.

Table 2 Genotype for the SNP1 marker of *TG* 5 gene in 41 Angus x Thai native crossbred heifers derived from 3 Angus sires.

Sires (Angus)	SNP1 genotypes of sires	SNP1 genotypes of heifer (Angus x Native)			Total
		CC	CT	TT	
AGQRFX 101	CT	2 (13.3%)	12 (80.0%)	1 (6.7%)	15
AGQRFX 015	CT	4 (36.4%)	7 (63.6%)	0 (0%)	11
AGQRFX 167	CC	14 (93.3%)	1 (6.7%)	0 (0%)	15
Total		20	20	1	41

Genotypic frequencies for the SNP1 marker of the TG 5 gene in different breed beef cattle were shown in Table 3. Frequencies of favorable allele T of the SNP1 marker of the TG 5 gene in 73 Angus x Thai native crossbred cattle was 40 and 1 % with SNP1 genotype of CT and TT, respectively.

Table 3 Genotypic frequencies for SNP1 marker of the *TG* 5 gene in different breed beef cattle

Breed	SNP1 genotype, %			Reference
	CC	CT	TT	
Wagyu	12	50	38	Nicol *et al*., 2001
Red Angus	42	42	16	Nicol *et al*., 2001
Black Angus	53	38	9	Nicol *et al*., 2001
Charolais x Angus	62	34	5	Van Eenennaam *et al*., 2007
Angus	64	32	3	Berendse *et al*., 2004
Hereford	81	18	1	Van Eenennaam *et al*., 2007
Brahman	95	4	1	Casas *et al*., 2005
Angus x Thai Native	59	40	1	This study

Discussion

An example to illustrate genotype is the single nucleotide polymorphism or SNP. A SNP occurs when corresponding sequences of DNA from different individuals differ at one DNA base, for example where the sequence AAGCCTA changes to AAGCTTA. This contains two alleles: C and T. SNPs typically have three genotypes. In the example above, the three genotypes would be CC, CT and TT. Genotypic and allele frequencies reported for each of the SNP examined in this study are summarized in Tables 1 and 2. These frequencies were derived from crossbred cattle and were therefore not necessarily reflective of any purebred population. They do give some indication as to the general prevalence of the favorable marker in Angus x Thai native crossbred cattle. These frequencies at least provide some indication as to the value of crossbreeding in Angus x Thai native crossbred cattle. The frequency of the favorable SNP1 genotype of TG 5 gene (TT) is greatest in the Wagyu breed, intermediate in other Bos taurus breeds, and lowest in Bos indicus breeds (Table 3). The Wagyu breed was well known in meat having highest marbling in the world. SNP1 genotypic frequencies of the TG 5 gene (TT) examined in Angus x Thai native crossbred cattle was closed to Hereford and Brahman breeds. Interestingly, SNP1 genotypic frequencies (CT) of Angus x Thai native crossbred cattle were higher than in other Bos taurus and in Brahman breeds. This is important because the potential impact of selecting for a genetic marker depends on both the magnitude of its effect and its frequency in the population.

In this study, two of 3 Angus sires (AGQRFX101, AGQRFX015) containing one copy of allele T (SNP1 genotype CT) increased the occurrence of 'CT' genotype (63.6 – 80.0%) in the Angus x Thai native crossbred cattle than an Angus sire (AGQRFX167) containing no copy of allele T (SNP1 genotype 'CC'). In order to breed steers with the favorable 'TT' SNP1 genotype, a bull and a cow which both have the 'TT' SNP1 genotype should be crossed. But if breeders are starting with stock in a minority of animals with 'CT' or 'TT' genotypes, it may take up to two generations of structured mating before large numbers of 'TT' genotype animals are on the herd. For breeding purposes, selecting animals which contain at least one copy (allele T) of the TG5 DNA fragment will increase the occurrence of the more favorable 'TT' genotype for marbling in beef cattle.

The favorable T allele of TG5 has been associated with increases in marbling score in both long-fed (>250 days on feed; Barendse, 1999), and short fed (<250 days on feed) Angus and Shorthorn animals where the genotype at this locus accounted for 6.5% of the residual variance for the marbling phenotype (Barendse *et al*., 2004). Three other peer-reviewed studies have examined associations of beef quality traits with the TG5 polymorphism. There was no association found between this marker and backfat in Bos taurus cattle (Moore *et al*., 2003), marbling score in Bos indicus cattle (Casas *et al*., 2005). 3.8% of animals with the 'TT' SNP1 genotype reached a marbling score five compared with 1.3% of those with the 'CC'.

Therefore, the TG 5 marker would be a candidate molecular marker for assisting selection of Angus sire to enhance possibility of marbling in Angus x Thai native crossbred cattle. Further study on evaluation of the association of this marker with marbling score

need to be taken in Angus x Thai native crossbred cattle. The TG 5 marker will be one of several SNP markers for marbling and be used for marker-assisted selection (MAS) in the crossbred beef breeding program.

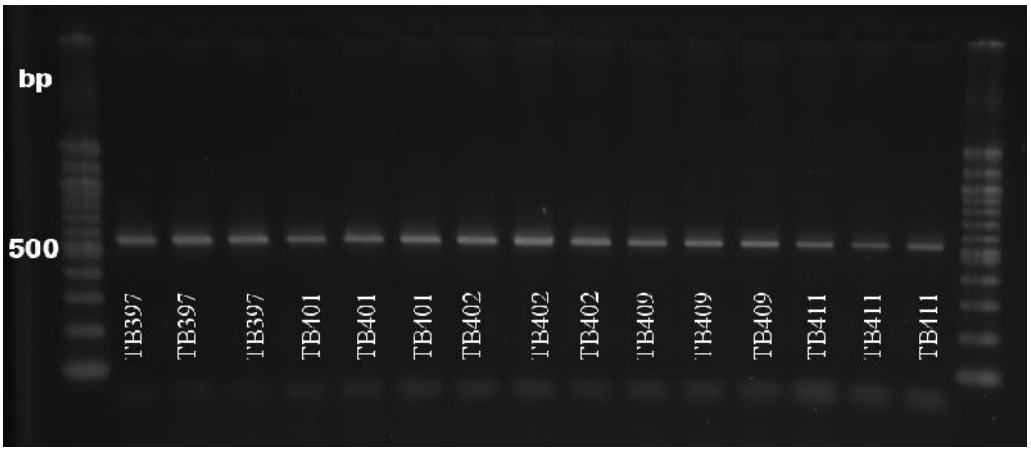

Figure 1 DNA fragments (537 bp) of the *TG* 5 gene of Angus x native crossbred cattle were amplified by PCR prior to DNA sequencing.

AAGCTTCCTGCTGCCTTTTGGTTGTCTGACGTCCTGGGACAGAGGGGAAAG GGGGATGAC 60

TACGAGTATGACTG TGCGTGTGTTTGGCTTATCTCATCAAAATCTCTACATTCTGTGTTA 120

ATGGATCTGCCTGTTTTGTTCCCTGCCATATCCTCATGGCCTAGAATAGTGTCTGCTTCT 180

CTATCAGACTCTAAAGAAACAT TGCTAGGAGGGAAGGAAGGAGCATGGATGAGGAGGGAG 240

GGAGCATTGTGTTTCTCTCACGGTGGGCCTGAACGTGTGGCCCACCAAGTTGTTAACTTT 300

GGCCTTTACCCCTGAAGATGAATTATGAAGCCACACCCCCAGTTCTTCCTTGGTGGCTCA 360

GATGGTCAAGAATCCACCTGCAATGCGGGAGACCTGGGTTTGATCCCTGGGTTGGGAAGA 420

SNP1

TCCCCTGGAGAAGGGAATGGCTACCCACTCCAGTATTCTGGCCTGGAGAATCCCATGGAC 480

AGAGGAGCCTGGCGGGATGCAGTCCATGGGGTCTCAGAGAGTCAGATGTGACTGAGCGAC 540

SNP2

TTTCACACACATTCGTCCCTGGTTCTGCTCCCCTACAGCCTCCACAAGATTTTCACCCCA 600

Figure 2 Nucleotide sequence at 5'-flanking region or 5'-untranslated region (5' UTR) of the *TG* 5 gene (GeneBank accession number X05380) indicated SNP1 and SNP2 at position 422 and 552. Primer sets were designed corresponding to the sequence in underlined letters.

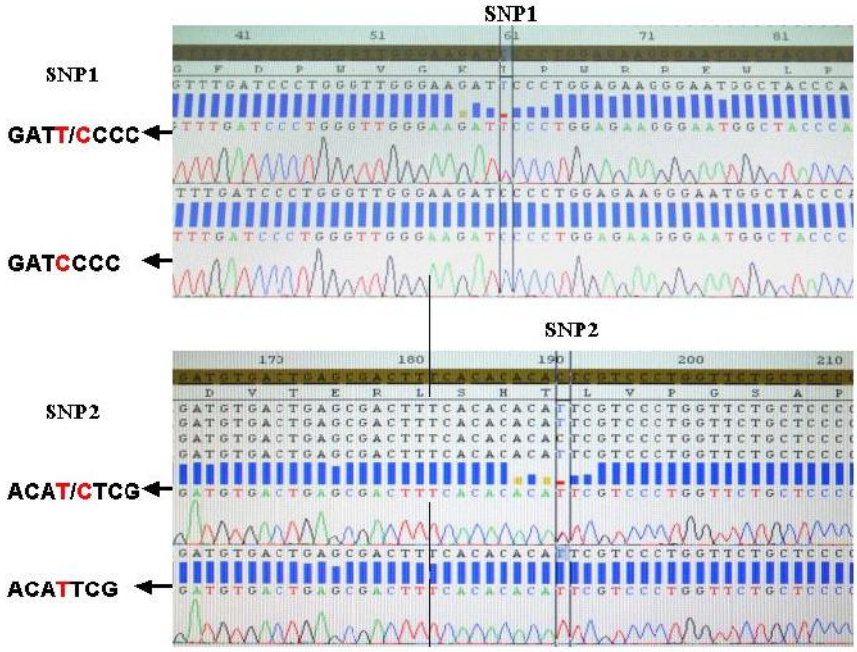

Figure 3 Single nucleotide polymorphism (SNP) of the *TG* 5 gene showing heterozygous (T/C) and homozygous (C or T) in the SNP1 and SNP2 positions

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สนิปจีโนไทป์ในยีนไธโรกลอบิวลินของโคลูกผสมพื้นเมืองไทย x แองกัส

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บทคัดย่อ

วัตถุประสงค์ในการศึกษาครั้งนี้เพื่อจำแนกจีโนไทป์ของนิวคลีโอไทด์ที่เปลี่ยนแปลง (SNP) ของยีนไธโรกลอบิวลิน (*TG*) ในโคลูกผสมพื้นเมืองไทย x แองกัส SNP ในยีนไธโรกลอ บิวลินเกิดขึ้นที่บริเวณ 5' ไพม์ของยีนนี้ซึ่งเป็นยีนที่ควบคุมการสร้างไกลโคโปรตีนสำหรับสร้างไธรอยฮอร์โมน มีความสัมพันธ์กับคะแนนไขมันแทรกเนื้อในโคเนื้อ คณะผู้วิจัยได้นำตัวอย่างดีเอ็นเอของโคลูกผสมพื้นเมืองไทย x แองกัสจำนวน 73 ตัว มาขยายชิ้นส่วนดีเอ็นเอของยีนส่วนนี้ด้วยปฏิกิริยาลูกโซ่โพลีเมอเรสก่อนนำไปหาการเรียงลำดับนิวคลีโอไทด์ ผลจากการวิเคราะห์พบว่ามีการเปลี่ยนแปลงของนิวคลีโอไทด์อยู่ 2 ตำแหน่งซึ่งตรงกับตำแหน่งนิวคลีโอไทด์ที่ 422 และ 552 ของยีนนี้ (GenBank accession number X05380) เป็น ไซโตซีน / ไธอะมีน (C/T) ที่สองตำแหน่งนี้ การจำแนกจีโนไทป์ของยีน *TG* 5 เป็น 3 จีโนไทป์คือ CC, CT และ TT ที่ 2 ตำแหน่ง (SNP markers) ผลจากการวิเคราะห์ตัวอย่างทั้ง 73 ตัวอย่างพบว่าความถี่ของจีโนไทป์ CC, CT และ TT ที่ SNP1 เท่ากับ 58.9, 39.7 และ 1.4 เปอร์เซ็นต์ ที่ SNP2 เท่ากับ 0, 68.5 และ 31.5 เปอร์เซ็นต์ ตามลำดับ มีรายงานการประเมินผลการใช้ SNP1 marker ของยีน *TG* 5 ว่าสัมพันธ์กับไขมันแทรกเนื้อในโคเนื้อสายพันธุ์ต่างๆ ซึ่งรูปแบบ(allele) ยีน *TG* 5 "T" สัมพันธ์กับคะแนนไขมันแทรกเนื้อในโคเนื้อพันธุ์แองกัส ดังนั้นคณะผู้วิจัยได้วิเคราะห์ตัวอย่างโคลูกผสมพื้นเมือง x แองกัส เพศเมียจำนวน 41 ตัวที่เกิดจากการนำน้ำเชื้อโคพ่อพันธุ์แองกัส 3 ตัวผสมกับแม่โคพื้นเมือง ปรากฏว่าลูกโคจำนวน 26 ตัวที่เกิดจากพ่อที่มีจีโนไทป์ CT ทั้ง 2 ตัว มีความถี่ของจีโนไทป์ CT สูง (63.6-80.0%) และมีความถี่ของ จีโนไทป์ TT ต่ำ (6.7%) ของลูกโคหนึ่งตัวใน 15 ตัวที่เกิดจากพ่อโคที่มีจีโนไทป์ CT นอกจากนี้ลูกโคจำนวน 15 ตัวที่เกิดจากพ่ออีกหนึ่งตัวที่มีจีโนไทป์ CC มีความถี่ของจีโนไทป์ CT ต่ำ (6.7%) จากผลการวิเคราะห์ครั้งนี้บ่งชี้ได้ว่าเครื่องหมายยีน *TG* 5 จะเป็นเครื่องหมายทางพันธุกรรมหนึ่งที่ช่วยในการคัดเลือกโคพ่อพันธุ์แองกัสเพื่อเพิ่มความเป็นไปได้ของการเพิ่มไขมันแทรกในเนื้อของโคลูกผสมพื้นเมืองไทย x แองกัส การประเมินผลความสัมพันธ์ระหว่างเครื่องหมายยีนนี้กับคะแนนไขมันแทรกเนื้อในโคลูกผสม พื้นเมืองไทย x แองกัส จำเป็นต้องทำการศึกษาต่อไป เพื่อยืนยันว่าเครื่องหมายยีน *TG* 5 จะเป็นหนึ่งในหลายๆ เครื่องหมาย SNP ของยีนอื่นๆที่สัมพันธ์กับไขมันแทรกเนื้อ และนำไปใช้เป็นเครื่องหมายทางพันธุกรรมที่ช่วยในโปรแกรมการคัดเลือกผสมพันธุ์ในโคเนื้อลูกผสมต่อไป

คำสำคัญ : แองกัส, โคลูกผสม, โคพื้นเมืองไทย, จีโนไทป์, สนิป, ไธโรกลอบิวลิน

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