Kalasin University Journal of Science Technology and Innovation
Volume: 4 Issue: 2 (July – December 2025)

Research Article

Genetic Variation and Polymorphism Identification of the Thyroglobulin (TG) Gene in Thai Wagyu Crossbred Cattle

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Date Received	Date Revised	Date Accepted
October 27, 2025	December 4, 2025	December 5, 2025

Abstract

The thyroglobulin (TG) gene is recognized as a major candidate gene involved in regulating intramuscular fat (IMF) deposition, a key determinant of beef quality. This study aimed to evaluate the genetic diversity and identify novel polymorphisms of the TG gene in Thai Wagyu crossbred cattle. Blood samples (n = 50) were collected from male animals to extract genomic DNA (gDNA). The target TG gene fragment was amplified using the polymerase chain reaction (PCR) and subsequently sequenced to identify single nucleotide polymorphisms (SNPs) and estimate population genetic parameters. Seven SNPs were identified including g.164A>G, g.257C>T, g.335A>G, g.386C>T, g.422C>T, g.537C>T, and g.552T>C. The expected heterozygosity (He) ranged from 0.41 to 0.49, while the polymorphic information content (PIC) ranged from 0.32 to 0.37, indicating moderate genetic diversity. The population was in Hardy–Weinberg equilibrium (HWE) ($P > 0.05$). The discovery of these seven novel SNPs provides essential baseline data for future association studies with meat quality traits and supports the potential application of TG gene polymorphisms as molecular markers in marker-assisted selection (MAS) programs aimed at improving meat quality in Wagyu crossbred cattle.

Keywords: Genetic variation, Thyroglobulin (TG) gene, Wagyu Crossbred Cattle

Introduction

High-quality beef production constitutes a fundamental goal of the global livestock industry, particularly in premium markets where superior sensory attributes such as tenderness, juiciness, and flavor are highly valued [1]. Among the most influential factors determining beef quality is intramuscular fat (IMF), commonly known as marbling, which significantly contributes to the organoleptic properties of meat [2]. The Wagyu breed is globally renowned for its exceptional genetic propensity to deposit IMF at substantially higher levels than other cattle breeds. Consequently, Wagyu genetics have been extensively integrated into crossbreeding programs to enhance carcass quality and market value in various cattle populations [3]. In Thailand, the premium beef sector has experienced rapid growth over the past decade, with Wagyu and Wagyu-crossbred cattle playing a pivotal role in meeting consumer demand for high-quality marbled beef. However, comprehensive studies on the genetic factors influencing marbling in Thai Wagyu-crossbred populations remain limited.

From an economic standpoint, the incorporation of Wagyu germplasm into commercial beef production has demonstrated notable profitability, with genetic improvements contributing to an 18–52% increase in economic returns compared to conventional systems [4]. This evidence underscores the substantial value of Wagyu and Wagyu-crossbred cattle as strategic assets in sustainable beef production.

Advances in molecular genetics have revolutionized animal breeding by enabling the identification of DNA markers associated with economically important traits. Among these, single nucleotide polymorphisms (SNPs) represent the most abundant, stable, and informative form of genetic variation across the genome. SNPs provide a robust tool for marker-assisted selection (MAS), allowing breeders to select genetically superior animals without the need for extensive phenotypic testing [5].

The thyroglobulin (TG) gene has emerged as a prominent candidate gene involved in lipid metabolism and fat deposition in beef cattle. It encodes a precursor protein essential for thyroid hormone synthesis, which plays a regulatory role in basal metabolic rate, lipogenesis, and adipocyte differentiation within muscle tissue [3]. Located on bovine chromosome 14 (BTA14), the TG gene is positioned within a quantitative trait locus (QTL) known to influence marbling and carcass quality traits [6].

Of particular interest is the TG5 polymorphism (g.-422C>T), located within the 5′-flanking region of the TG gene, which has been consistently associated with IMF accumulation in various beef breeds [3]. The TT genotype has been correlated with higher marbling scores, implying a positive regulatory effect on adipogenic gene expression, though the direction and magnitude of this association vary across cattle populations [6, 7]. Such variation is likely attributed to differences in genetic background, linkage disequilibrium, and environmental factors. Previous research on TG polymorphisms in other cattle breeds, such as Angus, Hereford, and Charolais, has further demonstrated breed-specific associations with IMF and carcass traits, emphasizing the need to evaluate whether these relationships hold in Thai Wagyu-crossbred populations. Including such comparative data highlights gaps in the current understanding and situates the present study within a broader genetic context.

In the context of Wagyu-crossbred cattle, understanding the allelic variation and functional effects of TG gene polymorphisms is critical. These populations often display enhanced meat quality while also possessing considerable genetic heterogeneity due to breed admixture [3]. Elucidating the genetic structure of TG polymorphisms in Thai Wagyu-crossbred cattle can provide practical markers for genomic selection, facilitating the accumulation of favorable alleles that improve carcass and meat quality traits.

All possible genotypic combinations were observed at each locus, including AA, AG, and GG for A>G; CC, CT, and TT for C>T; and TT, CT, and CC for T>C. Heterozygous genotypes (AG and CT) exhibited overlapping double peaks in the sequencing chromatograms, reflecting the simultaneous presence of both alleles, whereas homozygous genotypes showed single, well-defined peaks.

Although the TG5 polymorphism (g.–422C>T) has been extensively characterized across multiple cattle breeds and is widely recognized for its functional association with intramuscular fat (IMF) deposition, the broader landscape of TG gene variation in Thai Wagyu-crossbred cattle remains insufficiently documented. Previous studies have predominantly emphasized TG5 due to its consistent and biologically supported relationship with marbling traits. In contrast, other SNPs reported within the TG gene have not been consistently associated with IMF or carcass characteristics and often represent neutral sequence variations without confirmed phenotypic implications.

Identification of additional SNPs in Thai Wagyu-crossbred populations therefore addresses a critical knowledge gap by revealing previously undescribed or population-specific polymorphisms that may contribute to phenotypic variation in meat quality. Assessing the functional relevance of these newly detected SNPs is essential for determining their potential utility as molecular markers in future MAS strategies aimed at improving carcass and meat quality traits.

Objectives

To investigate the genetic variation and identify novel polymorphisms within the Thyroglobulin (TG) gene in a Wagyu crossbred cattle population.

Materials, Equipment, and Methods

Ethical approval

This study was conducted in compliance with the ethical standards for the care and use of animals in scientific research and was approved by the Institutional Animal Care and Use Committee (IACUC) of Kalasin University, Thailand (Approval No. KSU-AE-045, approved on May 1, 2025). All experimental procedures adhered to national and institutional animal welfare regulations.

Experimental animals and sample collection

A total of fifty (50) 50/50 Thai Wagyu–Angus crossbred bulls, aged between 18 and 24 months, were randomly selected from a private farm in Kalasin Province, Thailand. All animals were raised under a confined fattening system and were confirmed to be in good health at the time of selection. For each bull, 10 mL of whole blood was collected from the jugular vein using EDTA-coated vacuum tubes to prevent coagulation. The samples were immediately stored at 4 °C and transported to the laboratory within 24 hours for genomic DNA extraction.

DNA extraction and PCR amplification

Genomic DNA (gDNA) was extracted using the PureDireX® Genomic DNA Isolation Dual Kit (Column Based) (Gibthai Co., Ltd., Thailand) following the manufacturer's protocol. The purity and concentration of extracted DNA were determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Samples with an optical density (OD_{260}/OD_{280}) ratio between 1.8 and 2.0 were considered acceptable. The DNA integrity was further confirmed by electrophoresis on 1.0% agarose gels.

PCR Amplification and Sequencing

A DNA fragment encompassing the *TG* gene was generated through PCR amplification using the specific primer pair TG-F (5′-ggg gat gac tac gag tat gac tg-3′) and TG-R (5′-gtg aaa atc ttg tgg agg ctg ta-3′). These primers were designed based on the bovine *TG* gene sequence corresponding to GenBank accession number X05380 [8]. For the present study, the *TG* gene sequence used for primer design and comparative analysis was derived from publicly available reference data, while the genomic DNA template used for amplification originated from Thai Wagyu crossbred cattle sampled locally in Kalasin Province. The amplified fragments from these samples were subsequently sequenced de novo to confirm sequence identity and ensure compatibility with the reference *TG* gene sequence. Figure 1. PCR was carried out in mixtures with a final volume of 50 µl containing the forward primer (2.0 µl), reverse primer (2.0 µl), standard ready-to-use 2xPCR Mix (25 µl), DNA (4 µl) and nuclease-free water (17 µl). The PCR steps were carried out under the following temperature conditions: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, an annealing at 58°C for 45 seconds and elongation at 72°C for 45 seconds, then final elongation at 72°C for 5 minutes. The DNA fragment (537 bp) of TG gene were detected on 1.5 % agarose gel after staining with ethidium bromine through UV transillumination as shown in Figure 2.

DNA Sequencing and SNP Identification

The specific DNA fragment (537 bp) of TG gene of each sample were purified and submitted to Gibthai Co., Ltd. (Bangkok, Thailand) for bidirectional Sanger sequencing. Sequence quality was assessed using Sequence Scanner Software version 2.0, and multiple sequence alignment was performed with ClustalW implemented in BioEdit version 7.7 (https://bioedit.software.informer.com/). SNP positions were identified by aligning the sequences to the reference TG gene sequence of *Bos taurus* (GenBank accession number: JX090179.1).

Population Genetic Analysis

Genotypic and allelic frequencies were calculated for each SNP locus. The expected heterozygosity (He) was computed following Nei [6], while the polymorphic information content (PIC) and effective number of alleles (Ne) were determined according to Botstein et al. [9]. Hardy–Weinberg equilibrium (HWE) for each locus was assessed using the chi-square (χ^2) test at a significance level of $P < 0.05$.

Results and Discussion

A 537 bp DNA fragment of the TG gene was successfully amplified by PCR from the 50 Thai Wagyu crossbred cattle individuals (Figure 2), prior to DNA sequencing. Subsequent DNA sequencing was performed, and comparison with the reference sequence (GenBank accession number: X05380.1) revealed seven single nucleotide polymorphisms (SNPs) in the TG gene of the Thai Wagyu crossbred population. These identified SNPs include g.164A>G, g.257C>T, g.335A>G, g.386C>T, g.422C>T, g.537C>T, and g.552T>C. (Figure 1)

To enhance the clarity and interpretability of the results, it is recommended to include representative sequencing chromatograms illustrating both homozygous and heterozygous genotypes (e.g., AA versus AG or CC versus CT). Such figures would allow readers to clearly distinguish the single, discrete peaks characteristic of homozygous genotypes from the overlapping double peaks indicative of heterozygosity.

These findings confirm that multiple polymorphic loci exist within the TG gene region of the Wagyu crossbred population, suggesting a considerable degree of genetic variability that may influence economically important carcass traits.

Genotypic and Allelic Frequency Analysis of the TG Gene in Wagyu Crossbred Cattle

The genotypic and allelic frequencies of the seven identified SNPs are summarized in Table 1. The analysis revealed that all loci exhibited polymorphism, with expected heterozygosity (He) ranging from 0.41 to 0.49 and polymorphic information content (PIC) values ranging from 0.32 to 0.37, indicating a moderate level of genetic diversity within the studied population. Furthermore, all loci were in agreement with Hardy–Weinberg equilibrium ($P > 0.05$), suggesting that the population is genetically stable and not subject to selective pressure at the TG locus.

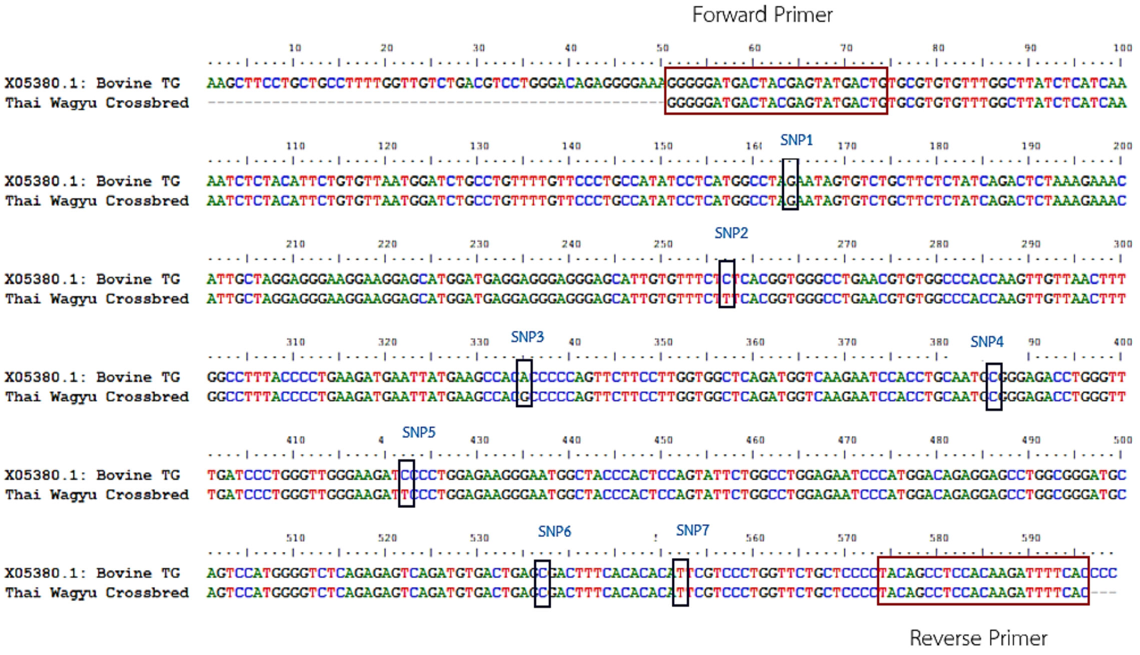

Figure 1. Comparison of the TG gene sequence fragment from Thai Wagyu crossbred cattle with the reference sequence (X05380.1). The alignment shows the position of the Forward and Reverse primers (boxed) and the locations of the seven single nucleotide polymorphisms (SNPs) identified in the study (SNP1 to SNP7).

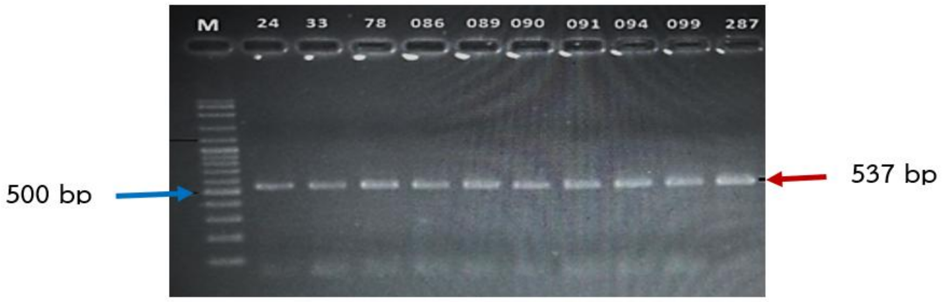

Figure 2 DNA fragments (537 bp) of the TG gene of Thai Wagyu crossbred cattle were amplified by PCR prior to DNA sequencing. M: 100bp DNA ladder as standard

Table 1 Genetic diversity of the TG genes in Thai Wagyu crossbred cattle compared with GenBank accession number X05380

SNPs	Genotype Frequencies (N=50)			Allele Frequencies		He	PIC	Ne	χ^2 (HWE)
g.164A>G	AA	AG	GG	A	G	0.47	0.36	1.89	0.72
	0.44	0.40	0.16	0.62	0.38				
g.257C>T	CC	CT	TT	C	T	0.49	0.37	1.96	0.81
	0.36	0.48	0.16	0.60	0.40				
g.335A>G	AA	AG	GG	A	G	0.41	0.33	1.69	0.65
	0.50	0.42	0.08	0.71	0.29				
g.386C>T	CC	CT	TT	C	T	0.48	0.36	1.90	0.79
	0.38	0.46	0.16	0.61	0.39				
g.422C>T	CC	CT	TT	A	G	0.46	0.35	1.86	0.68
	0.44	0.42	0.14	0.65	0.35				
g.537C>T	CC	CT	TT	C	T	0.41	0.32	1.68	0.71
	0.52	0.40	0.08	0.72	0.28				
g.552T>C	TT	CT	CC	T	C	0.43	0.34	1.73	0.74
	0.48	0.42	0.10	0.69	0.31				

N = Number of samples, He= Expected Heterozygosity, PIC = Polymorphic information content, Ne = Effective Number of Alleles, χ^2 (HWE) = Hardy-Weinberg equilibrium by the χ^2 -test ($\chi^2_{1;0.05} = 3.841$, $P<0.05$)

The thyroglobulin (TG) gene has been recognized as a promising genetic marker for the improvement of beef quality, particularly due to its influence on intramuscular fat (IMF) deposition, commonly evaluated through marbling score. Marbling is one of the most important traits affecting meat tenderness, flavor, and juiciness, and it varies significantly across different cattle breeds worldwide [10]. The biological function of the TG gene is closely linked to fat metabolism, as it encodes a precursor protein for thyroid hormones, which play a central role in regulating lipid metabolic pathways [11]. Several studies have demonstrated that genetic variations in the TG gene can directly influence the accumulation of intramuscular fat, thereby affecting marbling scores and overall meat quality [10, 11]. These findings highlight the potential of the TG gene as a valuable molecular marker for selective breeding programs aimed at enhancing beef quality traits, particularly in populations where marbling is a key determinant of market value.

Single nucleotide polymorphisms (SNPs) within the TG gene have been widely associated with marbling scores, notably the C422T SNP in the 5′ untranslated region (5′UTR) and several SNPs in the 3′ flanking region, including T354C, G392A, A430G, and T433G [10, 11, 12, 13, 14]. Cattle with TT or CT genotypes typically exhibit higher marbling than those with the CC genotype [15], though results vary across studies due to breed differences, population structure, and sample sizes [16].

In this study, we identified seven novel SNPs within the TG gene. Mapping these loci in relation to well-characterized variants, such as TG5, provides insights into their potential functional roles in intramuscular fat deposition. Linking our findings to previous studies not only contextualizes these new variants but may also help explain inconsistencies reported in earlier research.

The present study identified seven novel SNPs within the TG gene in Wagyu crossbred cattle, namely g.164A>G, g.257C>T, g.335A>G, g.386C>T, g.422C>T, g.537C>T, and g.552T>C, providing baseline genetic information and potential molecular markers for beef quality improvement through marker-assisted selection (MAS). While extensive research has established the TG5 polymorphism (c.–422C>T or g.422C>T) as a well-characterized marker strongly associated with marbling and intramuscular fat content in multiple cattle breeds [8, 10, 17, 18], the newly identified SNPs in this study expand the spectrum of TG gene variation and may offer additional predictive power. Notably, several of the novel loci are located in proximity to the TG5 site or within regulatory regions that could influence gene expression, suggesting potential functional relevance. For instance, g.422C>T corresponds to the TG5 locus, whereas the other six SNPs, located in both upstream and downstream regions, may interact or contribute additively to intramuscular fat deposition. This observation aligns with previous reports highlighting the context-dependent effects of TG polymorphisms, where genotype–phenotype associations can vary according to breed, population structure, and environmental conditions [16, 19].

By explicitly linking these novel SNPs to established TG5 data, the present findings provide a more comprehensive understanding of genetic variation within the TG gene in Wagyu crossbred populations. Future studies incorporating multi-locus analyses or functional assays could elucidate the additive or interactive effects of these SNPs, thereby enhancing the accuracy of MAS programs targeting marbling and overall meat quality. Ultimately, the identification of these novel variants reinforces the need to consider multiple genetic markers rather than relying on a single locus, consistent with recommendations for integrating genes related to lipid metabolism such as LEP and DGAT1 in predictive breeding strategies [13,16]

Conclusion

This study investigated the genetic diversity and identified novel polymorphisms within the Thyroglobulin (TG) gene in a Thai Wagyu crossbred cattle population. Analysis of 50 samples revealed seven novel Single Nucleotide Polymorphisms (SNPs): g.164A>G, g.257C>T, g.335A>G, g.386C>T, g.422C>T, g.537C>T, and g.552T>C. Population genetic analysis indicated a moderate level of genetic diversity, with expected heterozygosity (He) ranging from 0.41 to 0.49 and polymorphic information content (PIC) ranging from 0.32 to 0.37, and all loci conformed to Hardy–Weinberg equilibrium.

The discovery of these SNPs provides a valuable genetic baseline and potential functional insights, as variations in the TG gene may influence gene expression and intramuscular fat deposition, thereby affecting marbling and overall beef quality. The observed moderate genetic diversity suggests that the population retains sufficient variability for selective breeding, supporting the implementation of marker-assisted selection (MAS) strategies to enhance carcass traits.

These findings underscore the broader significance of TG gene polymorphisms as molecular markers in genetic improvement programs for Thai Wagyu crossbred cattle. Future research should focus on functional characterization of the identified SNPs, larger-scale validation in diverse populations, and the integration of these markers into breeding schemes aimed at optimizing meat quality and production efficiency.

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