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Research Article

Identification of Partial *GDF9* Gene for Genotyping Bligon Goats and Its Origin

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ABSTRACT

GDF9, a peptide in the *TGF-β* growth factor family, plays a crucial role in oocyte growth and maturation, affecting reproduction through genetic factors. This makes it valuable for livestock selection via SNPs, which are used in genotyping methods like PCR-RFLP. This study aimed to identify SNPs, map restriction enzyme sites, and perform genotyping to explore reproductive traits in Bligon goats. A total of 91 female goats were analysed, including 36 Bligon goats, 26 Peranakan Etawa goats, and 29 Kacang goats, and all of them had single births. PCR amplification was conducted using two pairs of primers for the *GDF9* gene: the forward primer 5'-CTCCTCTTGAGCCTCTGGTG-3' and reverse primer 5'-TCCAGTTGTCCCACTTCAGC-3' for GDF9-3, and forward primer 5'-TGTAAGATCGTCCCGTCACC-3' and reverse primer 5'-CACACTTCCTCTCCCTCTCA-3' for GDF9-6. Sequencing was done after amplification. Six SNPs were identified at positions g.2565C>M, g.2589G>A, g.2634G>A, g.2653G>T, g.2679A>G, and g.2691A>G, with one being synonymous (g.2653G>T) and five non-synonymous, causing amino acid changes. Among these, g.2565C>M was recognized by the restriction enzymes *HpaII* and *MspI*, was recommended for use in PCR-RFLP for genotyping with the *GDF9*-3 primer. The study also found that the genotype frequencies in Bligon, Peranakan Etawa, and Kacang goats were in Hardy-Weinberg equilibrium (HWE), supporting the use of SNP g.2565C>M as a genetic marker. Enzym *MspI* can be used for genotyping the target gene in exon 2 of the *GDF9* gene in three types of goats: Bligon, Peranakan Etawa, and Kacang.

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INTRODUCTION

Efforts to enhance goat productivity focus on improving reproductive traits, such as rapid sexual maturation, high litter sizes, and short kidding intervals. Bligon goat breed known for these traits as a result from crossbreeding of Peranakan Etawa (PE) and Kacang goats and is characterized by its triangular-shaped muzzle, hanging ears, and compact body. These goats are hardy, adaptable, and easy to maintain (Sarwono 2011). Reproductive traits in goats, such as ovulation rate, are influenced by inheritable genes, which can be targeted for improvement through molecular methods (Ginting 2009). Genes like *BMPR1B*, *GDF9*, *BMP15*, *FSHβ*, *FSHR*, *POU1F1*, *PRLR*, and *KISS1* are known to affect reproduction in goats (Ahlawat et al. 2015a). Among them, Growth Differentiation Factor 9 (*GDF9*) plays a vital role in ovulation and oocyte maturation. *GDF9*, produced by oocytes and part of the TGF-β Growth Factor family, is located on chromosome 7, spanning 2 exons and 1 intron, with a total length of about 5644 bp (Arta & Rahayu 2013; Chairunissa et al. 2022).

The *GDF9* gene plays a critical role in controlling reproductive traits, particularly in regulating the number of offspring produced in each cycle (Jia et al., 2015). The number of offspring is influenced by the quantity of eggs ovulated, which is tied to Follicle Stimulating Hormone (FSH) secretion during folliculogenesis. *GDF9* stimulates granulosa cell differentiation, induces receptors for Luteinizing Hormone (LH), and promotes steroidogenesis. Found in both oocytes and granulosa cells, *GDF9* triggers the proliferation of granulosa and cumulus cells before and during ovulation (Arta & Rahayu 2013). A deficiency in *GDF9* impairs follicle growth and granulosa/theca cell function, leading to infertility.

GDF9's influence on reproduction is a polygenic trait, making it valuable for livestock breeding. The use of Single Nucleotide Polymorphisms (SNPs) as genetic markers enhances breeding efficiency (Prihandini & Maharani 2019). SNPs are single base changes that help identify inheritable traits and are commonly used in molecular breeding. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) is a reliable method for detecting SNPs linked to desirable traits in animals (Wang et al. 2021). This method allows for precise genetic selection and marker-assisted breeding (Liu & Chen 2020) and has been shown to improve selection accuracy and genetic mapping in agricultural species (Kim et al. 2023). Combining SNP analysis with PCR-RFLP has great potential to boost livestock productivity through targeted genetic improvement programs.

PCR-RFLP with the *BsaI* enzyme has been applied in studies like Ghoreishi et al. (2019), where polymorphisms in the *GDF9* gene in Markhoz goats were linked to prolificacy. This study identified three genotypes (CC, CA, AA) with different fragment sizes, demonstrating how restriction enzymes can effectively identify genetic markers and provide insights into traits linked to mutations (Perdana & Hartatik 2022). A study by Hartatik et al. (2023) demonstrated the use of restriction enzymes *BsaI* and *BsmAI* at the g.1956A>C SNP in Exon 1, while *MspI*, *HapII*, and *HpaII* were used to recognize the g.3855A>C SNP in Exon 2 based on GenBank Acc. No. EF446168. Based on this, Irawan et al. (2024) reported that genotyping with PCR-RFLP at the g.3855A/C position in Exon 2 of the *GDF9* gene revealed two genotypes, AA and AC, using the *MspI* enzyme. The *GDF9* gene is a key candidate for traits like litter size, which is important in animal breeding. However, no studies have yet identified specific sequences in Exon 2 using two primers or compared them with the genetic sequences of origin goats. This gap highlights the need for further research, particularly studies on mutation analysis (SNPs and amino acid changes), restriction enzyme mapping, and genotyping of the *GDF9* gene in Bligon goats and their origin breeds, such as PE and Ka-

cang goats. Exploring these areas could provide valuable insights into the genetic basis of litter size and other economically important traits in goats.

MATERIALS AND METHODS

Bloods samples

The materials used in this study consisted of isolated blood samples from 91 animals, consist of 36 Bligon goats, 26 PE goats, and 29 Kacang goats, all of them were female and selected based on single birth type. Approximately one -third of the neck's distal area was obstructed, and blood was collected from the jugular vein. To prevent any contamination during the blood sampling process, a venoject connected to a K3EDTA tube was utilised, and the constrained area was sterilised using alcohol. The venoject was inserted into the blood vessel at an upward angle of 30°, resulting in the collection of a total of 3 ml of blood. The blood stored in the K3EDTA tube was transported to the laboratory while maintaining a low temperature inside an icebox containing ice packs. The blood sampling protocol employed in this study received approval under Ethical Clearance Number 0124/EC-FKH/Eks/2022.

DNA extraction

The DNA extraction was performed following the instructions of the DNA isolation kit (gSYNCTM DNA Extraction Kit, Geneaid, Taiwan) at the Laboratory of Genetics and Animal Breeding, Faculty of Animal Science, Universitas Gadjah Mada.

DNA amplification of GDF9-3 and GDF9-6

The genomic DNA of 91 samples were amplified using PCR method using two sets of primer. The first primer is GDF9-3 (forward primer 5'-CTCCTCTTGAGCCTCTGGTG-3' and a reverse primer 5'-TCCAGTTGTCCCACTTCAGC-3'). The PCR reaction mix were 25 µL total volume, including 12.5 µL of PCR Kit My Taq HS Red Mix (Meridian Bioscience, US), 9.5 µL of DDW, 2 µL of DNA with total 20 ng, and 0.5 µL of each primer with the concentration of 10 pmol μL^{-1}. With the PCR program as follow: pre-denaturation step at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 40 seconds, and extension at 72°C for 30 seconds. The final extension was at 72°C for 5 minutes. The second primer set, GDF9-6 (forward primer 5'-TGTAAGATCGTCCCGTCACC-3' and a reverse primer 5'-CACACTTCCTCTCCCTCTCA-3') with similar method as previous primer. The PCR products were then separated by electrophoresis on a 1% agarose gel at 50 volts for 25 minutes.

GenBank accession number EF446168.2 and OP494705.1 were used as reference for the *GDF9* gene target sequence and the primers location scheme were shown in Figure 1.

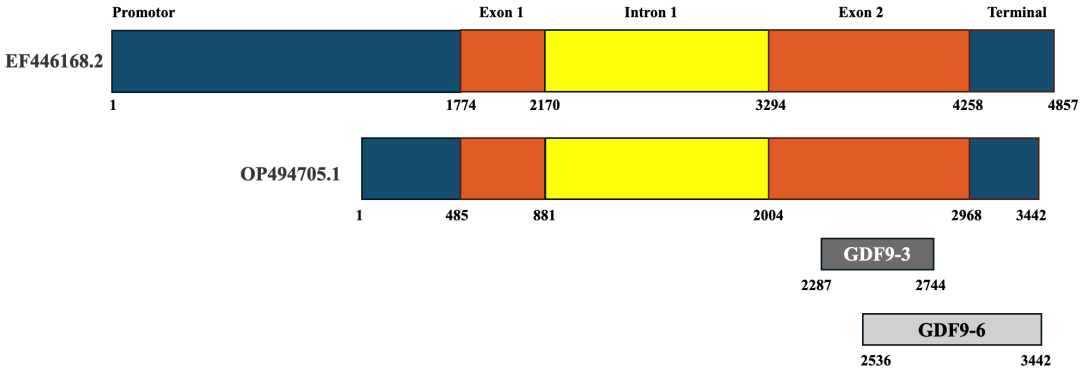

Figure 1. GenBank references EF446168.2 and OP494705.1 of *GDF9* gene and target sequence of two pairs of primers (GDF9-3 and GDF9-6).

Sequencing and SNP identification of targets

The Sanger sequencing process was conducted in LPPT UGM (Yogyakarta, Indonesia). A set of 25 µL of PCR product along with 10 µL of primers was delivered to LPPT, and the results were then aligned using BioEdit software version 7.2.5. SNPs were detected from an electropherogram that showing homozygous and heterozygous sequences. Homozygous SNPs are indicated by a single peak, while heterozygous SNPs are indicated by double peaks. The sequencing approach provides a more accurate confirmation of SNPs, which is then followed by PCR-RFLP analysis for further validation (Figure 3). The sequencing result then were being aligned with the GenBank reference.

Restriction enzyme determination and mapping

We used NebCutter V2.0 website to identify restriction enzymes as well as the restriction site of the SNP. The identification of specific restriction enzymes was able to recognize gene targets and was indicated by the appearance of a red line beneath a sequence (Albakri & Hartatik 2021). The count of cleavage sites was determined using BioEdit v.7.2.5, applied to both the GenBank samples and references.

Genotyping restriction fragment length polymorphism (RFLP)

After identifying the appropriate restriction enzyme from the mapping results, the PCR-RFLP phase was carried out. The SNP g.2565C>M from 91 samples was used for genotyping using PCR-RFLP method. Restriction enzyme digestion was performed in 12 µL reaction volumes, consisting of approximately 3 µL of PCR product with a total of approximately 150 ng DNA, 7.7 µL of ddH2O, 1.2 µL of Buffer 1.1 (New England Biolabs, USA), and 0.1 µL of *MspI* restriction enzyme (10 U μL^{-1}; 1 Unit total). The digestion was incubated for 2 hours at 37°C. The digested products were then analysed by running them on a 3% agarose gel, 50 Volt, and for 30 minutes.

Data analysis

The data analysis employed is descriptive, involving the grouping of alleles and genotypes from the PCR-RFLP results. The allele (1) and genotype (2) frequency analysis can be determined using the formula as follow:

(1) Formula allele frequency

$$X_i = \frac{2N_{ii} + N_{ij}}{2N}$$

$$X_j = \frac{2N_{jj} + N_{ij}}{2N}$$

(2) Formula genotype frequency

$$X_{ii} = \frac{N_{ii}}{N} \times 100\%$$

$$X_{jj} = \frac{N_{jj}}{N} \times 100\%$$

$$X_{ij} = \frac{N_{ij}}{N} \times 100\%$$

Where X_i = frequency of allele i, X_j = frequency of allele j, X_{ii} = genotype ii, X_{jj} = genotype jj, X_{ij} = genotype ij, N = number of individual samples.

Pearson's Chi-square test was used to verify the Hardy–Weinberg equilibrium status for the allele and genotype frequencies using following mathematical model:

$$X^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

Where X^2 is the Chi-square test value, O_i is the observed frequency, E_i is the expected frequency.

RESULTS AND DISCUSSION

Identification of SNPs *GDF9* gene using GDF-3 and GDF-9 primers

The PCR product using GDF9-3 and GDF9-6 primers were 456 bp and 907 bp, respectively as shown in Figure 2A. Based on the results, four SNPs position were found in GDF9-3 (SNPs: g.2565C>M, g.2589G>A, g.2634G>A, and g.2679A>G) spanned throughout exon 2 (Figure 2B) and three SNPs position were found in GDF9-6 (SNPs: g.2653G>T, g.2679A>G, and g.2691A>G) spanned in exon 2 and the terminal region (Figure 2C). Sequence alignment and electropherograms confirmed the SNP positions, heterozygous from homozygous sequences.

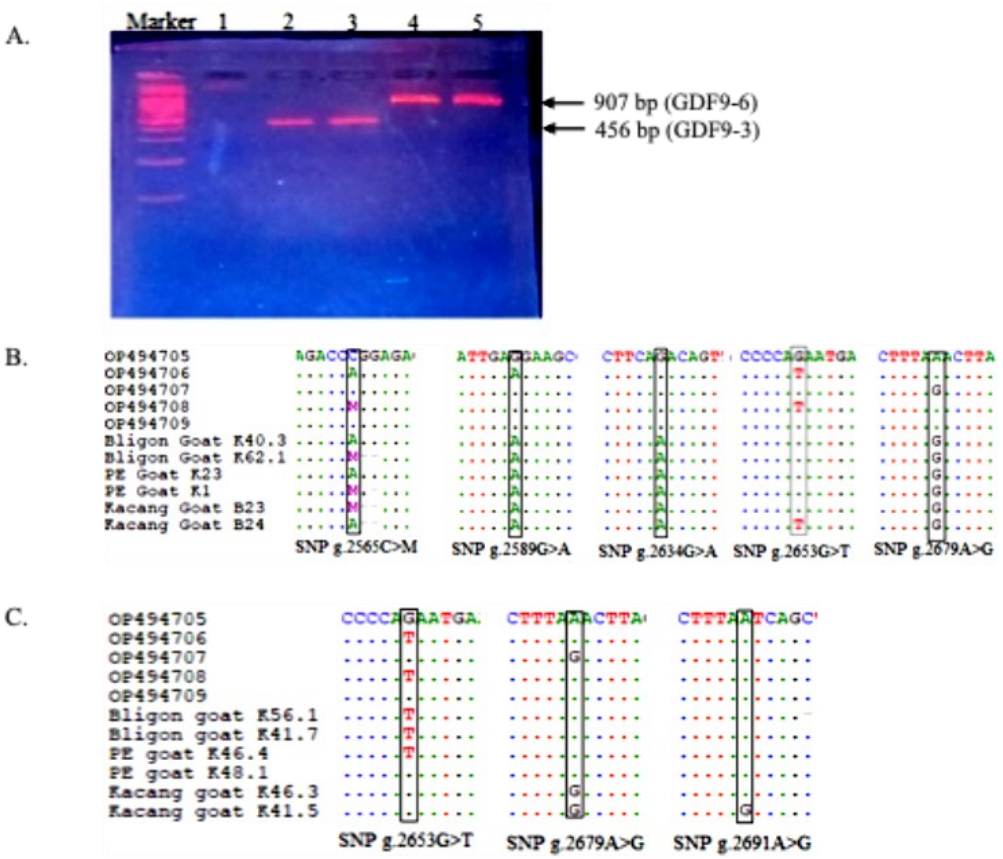

Figure 2. Product size of PCR and alignment results from sequencing in *GDF9* target gene of Bligon, PE, and Kacang goat. Note: A = PCR product (Marker 100 bp), B = GDF9-3 sequence alignment results, and C = GDF9-6 sequence alignment results.

The electrophoregram analysis provides significant insights into the genetic variations within the *GDF9* gene in the studied population. SNP at position g.2565C>M exhibited two genotypes: AA and AC. The AC genotype showed double peaks at both A and C bases, indicating heterozygosity. In contrast, the SNP at position g.2589G>A presented only the AA genotype, characterized by a single peak at base A, indicating homozygosity for the A allele. We have found SNPs at g.2634G>A for Bligon goat found two genotypes (GG and AA), while in the other goats (Kacang and PE) only one genotype (AA). Furthermore, the SNPs at g.2679A>G for Bligon goat found two

genotypes (AA and GG), while in the other goats (Kacang and PE) only one genotype (GG) found in this study. These findings are consistent with Chairunissa et al. (2022) who reported the SNP g.3855A>C (with GenBank reference number EF446168.2) that we found the SNPs at SNP g.2565C>M (with GenBank reference number OP494705.1 in the three local goats in our study). It means that this highlighting the reliability of these genetic markers and their potential utility in breeding programs.

While three SNPs g.2653G>T, g.2679A>G, and g.2691A>G were identified, each displaying two genotypes: TT/GG for g.2653G>T, AA/GG for g.2679A>G, and AA/GG for g.2691A>G were flanked by primer GDF9-6. These genotypes were marked by distinct peaks for bases T/G, A/G, and A/G, respectively, in the electropherogram. These findings are aligned with Zhu et al. (2013), who observed similar SNP variations in different goat breeds, highlighting the functional relevance of these genetic markers across livestock populations. The results have important implications for Marker-Assisted Selection (MAS) in breeding programs. The consistent identification of these SNPs in various studies underscores their potential as reliable markers for selecting animals with desirable traits, especially reproductive traits like litter size. Supporting this, Wouobeng et al. (2020) found that the AA genotype in Markhoz goats was associated with larger litter sizes, and Feng et al. (2011) discovered similar SNP variations in Jining Gray goats, further validating the role of these genetic markers in improving reproductive outcomes.

These findings not only confirm the presence of specific SNPs in the *GDF9* gene but also contribute to improving livestock breeding strategies. The consistent genotyping results from this study, alongside those of Zhu et al. (2013) and Feng et al. (2011), enhance the understanding of how these genetic markers can be utilized to select for reproductive traits, a crucial aspect of livestock management and genetic improvement.

The amino acid analysis revealed one synonymous (nonsense) mutation and five non-synonymous (missense) mutations. The SNP at position g.2565C>M exhibited both homozygous CC and heterozygous AC genotypes. The SNP g.2589G>A resulted in an amino acid substitution, changing Glycine to Arginine. Similarly, the SNP g.2634G>A caused an amino acid change from Aspartic acid to Asparagine. The SNP g.2653G>T led to a premature stop codon (nonsense mutation), while the SNP g.2679A>G resulted in an amino acid change from Asparagine to Aspartic acid. Finally, the SNP g.2691A>G resulted in an amino acid change from Isoleucine to Valine. These amino acid changes are summarized in Table 1.

The SNP g.2565C>M, for example, results in a mutation from Glutamine to Proline, which, although missense, may still affect protein structure and function. Such mutations can influence protein folding, stability, and activity, which in turn may impact the function of *GDF9* a key growth differentiation factor involved in reproductive processes in animals (Zhu et al. 2013). This is consistent with findings from Wang et al. (2018), who noted that SNPs in the *GDF9* gene could alter its expression and functionality, potentially leading to variations in reproductive performance across livestock populations.

Other studies have further explored the role of *GDF9* SNPs in reproductive traits. Ghoreishi et al. (2019) identified a SNP in Markhoz goats (g.183C>A) that did not alter the amino acid Leucine, while Du et al. (2008) found a heterozygous genotype (g.1189G>A mutation) in Guizhou white goats. Among a group of 33 high-productivity brood goats, those with the heterozygous genotype produced three offspring per birth, compared to low-productivity goats with a homozygous genotype. Aboelhassan et al. (2021) also highlighted that *GDF9* gene polymorphism influences fecundity, with

Table 1. Results of amino acid changes in the *GDF9* gene of Bligon, PE, and Kacang goats.

No	SNP Position (OP494705)	Codon	Amino Acid	Mutation	Primer	SNPs status		
						Bligon	PE	Kacang
1	g.2565C>M	CAG CCG	Glutamine Proline	Missense mutation	GDF9-3	Yes	Yes	Yes
2	g.2589G>A	GGA AGA	Glycine Arginine	Missense mutation	GDF9-3	Yes	No	No
3	g.2634G>A	GAC AAC	Aspartic acid Asparagine	Missense mutation	GDF9-3	Yes	No	No
4	g.2653G>T	GAA UAA	Glutamic acid Stop	Nonsense mutation	GDF9-6	Yes	Yes	No
5	g.2679A>G	AAC GAC	Asparagine Aspartic acid	Missense mutation	GDF9-3 and GDF9-6	Yes	No	No
6	g.2691A>G	AUC GUC	Isoleucine Valine	Missense mutation	GDF9-6	No	No	Yes

heterozygous genotypes leading to higher ovulation rates and larger litter sizes in livestock compared to homozygous genotypes in wild populations.

Several previous studies confirmed that missense mutation was found in exon 2 of the *GDF9* gene, such as Hartatik et al. (2023), found SNP g.3855A>M (position from reference EF446168.2) in Bligon goats with an amino acid change from Glutamine to Proline, Zhao et al. (2016) identified SNP g.3905A>C in Kashmir goats, which caused an amino acid change from Valine to Isoleucine, and Feng et al. (2011) detected SNP g.397IG>A in Jining Grey goats, resulting in a Glycine to Aspartic acid change in exon 2 of *GDF9* gene. These studies underscore the importance of identifying SNPs in the exon regions, as they can lead to amino acid changes that may influence reproductive performance, providing valuable information for livestock breeding programs (Perdana 2022).

Restriction enzyme mapping of *GDF9-3* and *GDF9-6*

The restriction enzyme mapping for SNPs in the *GDF9* gene revealed key enzymes suitable for PCR-RFLP genotyping, specifically *MspI* and *HpaII* for SNP g.2565C>M, and *Hpy188I* and *MseI* for SNPs g.2679A>G and g.2691A>G, respectively. *MspI* and *HpaII* enzymes, found at position g.2565C>M, present a unique restriction site with only one cutting site, enabling the identification of three genotypes: CC, AA, and AC. The corresponding fragment sizes for the genotypes are as follows: the AA genotype produces a 456 bp fragment; the AC genotype produces three fragments (456 bp, 307 bp, and 149 bp); and the CC genotype produces two fragments (307 bp and 149 bp). These results make *MspI* an ideal restriction enzyme for genotyping, as highlighted in Table 2.

Restriction enzyme mapping is a useful tool for genetic tagging, as it identifies SNP-associated restriction sites and enables accurate genotyping (Perdana & Hartatik 2022). The use of *MspI* and *HpaII* enzymes is supported by their ability to generate distinct, well-spaced fragment sizes, essential for clear visualization and differentiation of genotypes. The enzymes' specificity is also beneficial, as each enzyme recognizes a single restriction site within the SNP regions. These findings align with earlier studies by Hossain et al. (2020) and Chairunissa et al. (2022), who also used restriction enzymes such as *HhaI*, *DdeI*, and *MspI* for genotyping *GDF9* gene polymorphisms. Furthermore, Hartatik et al. (2023) identified multiple enzymes (*MspI*, *HpaII*) as suitable markers for further genotype identification using PCR-RFLP methods.

The choice to utilize *MspI* and *HpaII* in this study was based on several practical considerations, including their ability to generate sufficiently sized

Table 2. Mapping of restriction enzymes on target *GDF9-3* and *GDF9-6* of Bligon, PE, and. Kacang goat.

Target	Product size	SNP	Enzyme	Recognizing site	Amount of cut	Site of cut	Fragment Size
GDF9-3	456	g.2565C>M	*MspI* *HpaII*	C'CG_G	1 cutter	307	307, 149
GDF9-6	907	g.2679A>G	*Hpy188I*	TC_n'GA	5 cutters	17, 54, 68, 499, 757	17, 37, 14, 431, 258, 150
GDF9-6	907	g.2691A>G	*MseI*	T'TA_A	10 cutters	111, 123, 399, 608, 704, 712, 775, 825, 829, 843	111, 12, 276, 209, 96, 8, 63, 50, 4, 14, 64

bands (over 100 bp), the clear separation of band sizes for easy visualization, and the availability of these enzymes in the lab. In comparison, enzymes like *MseI* and *Hpy188I*, which produce smaller fragment sizes, were less optimal for this study. Overall, the identified restriction enzymes offer reliable markers for genetic analysis and potential applications in livestock breeding programs.

Polymerase chain reaction-restriction fragment length polymerase (PCR -RFLP) of Bligon, PE, and Kacang goats using GDF9-3

The findings from the PCR and PCR-RFLP analysis of the *GDF9* gene are presented in Figure 3, highlighting the genotyping of SNP g.2565C>M in 91 goats using the *MspI* enzyme, which identifies the C|CGG restriction site. The PCR-RFLP analysis identified two distinct genotypes: the homozygous AA genotype, characterised by a 456 bp fragment, and the heterozygous AC genotype, which showed fragments of 149 bp, 307 bp, and 456 bp.

For Bligon goats, most animals exhibited the AA genotype (0.97 frequency), with a smaller proportion showing the AC genotype (0.03). The A allele had a frequency of 0.98, while the C allele had a frequency of 0.02. The Hardy-Weinberg equilibrium (HWE) test for Bligon goats yielded a chi-square (χ^2) value of 0.14, indicating equilibrium. Similarly, in PE goats, the AA genotype was the most common (0.96 frequency), followed by the AC genotype (0.04). The A allele frequency was 0.98, and the C allele frequency

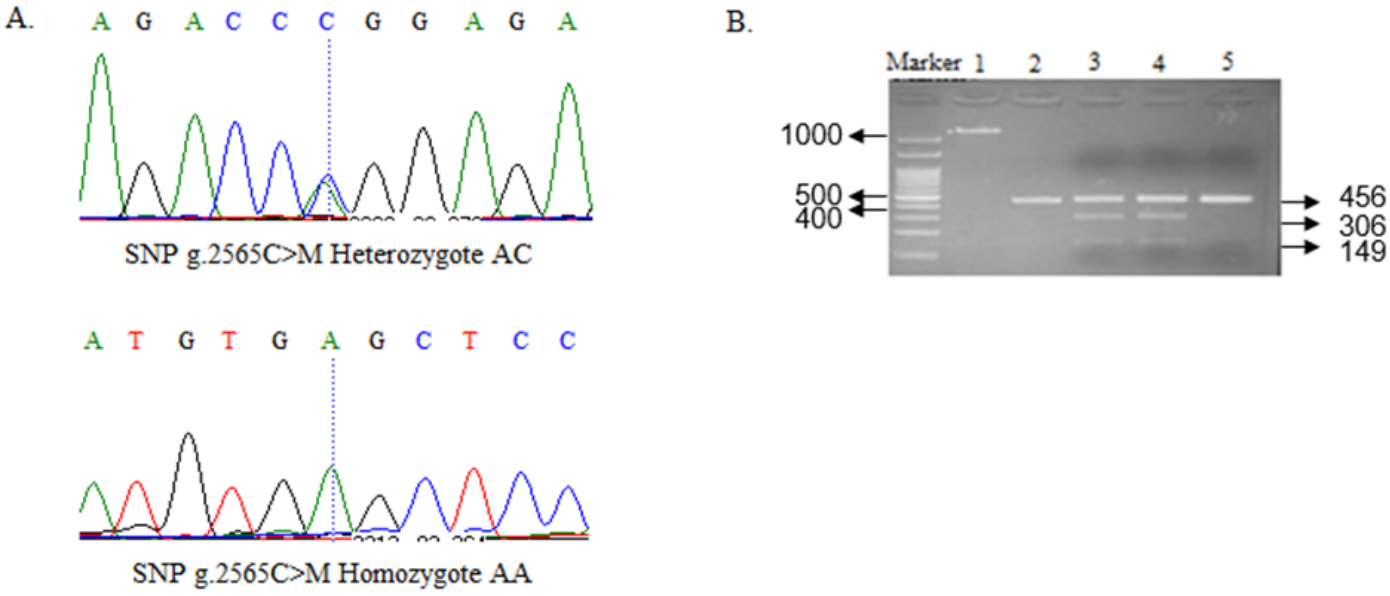

Figure 3. Chromatogram SNP g.2565C>M and PCR-RFLP with *MspI* enzyme. Note: A = Chromatogram of SNP g.2565C>M Heterozygote AC and Homozygote AA, B = Product size of PCR-RFLP in *GDF9* target gene of Bligon, PE, and Kacang goat. 1: DNA isolation, 2: Bligon goat, 3: Bligon goat (K62.1), 4: PE goat (K1), and 5: Kacang goat (B24) (Marker 100 bp, AA= genotype homozygote, AC= genotype heterozygote).

was 0.02, with a chi-square (χ^2) value of 0.01 for HWE. In Kacang goats, the AA genotype frequency was 0.69, while the AC genotype occurred in 0.31 of the animals (Table 3). The A allele had a frequency of 0.84, and the C allele had a frequency of 0.16, with a chi-square (χ^2) value of 0.28 for HWE.

The allele frequencies observed in the populations indicated polymorphism, as the frequency of the common C allele was lower than 0.99, confirming the polymorphic nature of the goat population.

Individuals with the AA genotype exhibited a fragment size of 456 bp, while homozygous AC individuals showed three fragment sizes: 456, 307, and 149 bp. The results from Pearson's Chi-square test indicated that the genotypes did not significantly deviate from Hardy-Weinberg equilibrium (HWE) ($P > 0.05$). Based on Hardy-Weinberg principles, this suggests that the allele and genotype frequencies in the population will remain stable across generations, provided there are no external influences, such as selection, mutations, migration, or inbreeding, that could affect the population randomly (Warwick et al. 1990).

The *MspI* restriction enzyme was used to identify genotypes in goats at position g.2565C>M. Previous studies by Feng et al. (2011), Ahlawat et al. (2015b), Chairunissa et al. (2022), and Hartatik et al. (2023) found two genotypes, AA and AC, in goats using *MspI*. The AA genotype showed a 456 bp fragment, the AC genotype showed fragments of 456, 307, and 149 bp, and the CC genotype showed 307 and 149 bp fragments. In this study, Bligon, PE, and Kacang goats showed only AA and AC genotypes, with AA being the most common. The absence of the CC genotype may be due to the small goat population in the study. The HWE test indicated that the genotypes in these populations were balanced, suggesting no issues like selection, mutation, migration, or inbreeding. More research is needed to explore the potential link between genotype and litter size in these goat populations.

CONCLUSIONS

Based on the research conducted, the restriction enzymes recommended for the target sequence SNP g.2565C>M are *HpaII* and *MspI*. PCR-RFLP analysis showed that only two genotypes (AA and AC) were found in Bligon, PE, and Kacang goats. Additionally, the AA genotype had the highest frequency compared to the heterozygous AC genotype. The HWE analysis showed that the genotype distribution of the *GDF9* gene in the Bligon, PE, and Kacang goat populations is stable. In conclusion, the *MspI* restriction enzyme can be used for genotyping the target gene in exon 2 of the *GDF9* gene in three types of goats: Bligon, PE, and Kacang.

AUTHOR CONTRIBUTION

F.J.F., D.P.S., N.P.N., F.A.C., and A.I. conducted the research and wrote the

Table 3. Genotype frequency, allele frequency, and Hardy–Weinberg equilibrium in Bligon, PE, and Kacang goats.

	Genotype (SNPs g.2565C>M)	N	Genotype frequency	Allele frequency	X^2
Bligon	AA	35	0.97	A = 0.98	
	AC	1	0.30	C = 0.02	0.14
	CC	0	0.00		
PE	AA	25	0.96	A = 0.98	
	AC	1	0.40	C = 0.02	0.01
	CC	0	0.00		
Kacang	AA	20	0.69	A = 0.84	
	AC	9	0.31	C = 0.16	0.28
	CC	0	0.00		

Note: X^2 value for a 0.05 significance level with df = 1 is 3.841.

manuscript. M.P.R., K., S.B., and T.H. performed data analysis and supervised the manuscript.

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CONFLICT OF INTEREST

The authors have no conflicts of interest related to the research or the funding support for this study.

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