Restriction Enzyme Mapping of MC4R Gene in Bligon Goat Using Bioedit Program

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ABSTRACT

Restriction enzymes are a fundamental tool of molecular biology for determination of diversity and in vivo function. Many research used restriction enzyme for genotyping mammals based on MC4R gene. The objective this study to map the restriction enzyme of MC4R gene in Bligon goat. The mapping of restriction enzyme on MC4R gene was carried in Bligon goat (n=10). MC4R gene was amplified using specific primer forward and reverse, resulting 642 bp of amplification products. We discovered 5 enzymes (*FatI, NlaIII, RsaI*, *Acc651* and *KpnI*) which recognized 2 SNPs region based on restriction mapping using Bioedit program. There were three recommended enzymes (*RsaI*, *Acc651* and *KpnI*) for genotyping the Bligon goat. In the present study, an individual with CC genotype produced 2 fragments with enzyme *RsaI* and 1 fragment with *Acc651* and *KpnI.* An individual with CT genotype produced 4 fragments with enzyme *RsaI* and 3 fragments with *Acc651* and *KpnI.* In conclusion, we suggested that restriction enzymes *RsaI, Acc651* and *Kpn1* may be used for genotyping of a targeted gene using PCR-RFLP method for Bligon goat in the future research.

Keywords: Bligon goat, Sequencing, MC4R gene, restriction mapping, Bioedit.

INTRODUCTION

A restriction enzyme is a fundamental tool of molecular biology for determination of diversity and in vivo function. An understanding of the enzymes and their properties can improve their productive application by maintaining critical digest parameters and enhancing or avoiding alternative activities (Williams, 2003). BioEdit is one of the most common programme used in molecular biology analysis. It was developed initially as a biological sequence alignment editor written for Windows only (Hall, 2011). BioEdit features include automated ClustalW alignment, RNA comparative analysis tools, translation based nucleic acid alignment and restriction enzyme mapping. Many researchers in the field of molecular biology have used BioEdit modules in during their original research BioEdit was used for molecular studies in virus genomes (Chen *et al*., 2006), Bacterial genome (Aquifer *et al*., 2003), plant genome (Klaas *et al*., 2002) and animal genome (Hong et al., 2008). The MC4R gene have been reported by researchers that used the restriction enzymes for genotyping in cattle (Liu *et al.,* 2010), sheep (Wang *et al.,* 2015), pig (Piorkowska *et al*., 2010; Kim *et al*., 2000), dog and red fox (Skorczyk *et al*., 2007) and chicken (Zhou *et al*., 2012). However, no report on the restriction enzymes of MC4R gene study have been identified in Bligon goat. Therefore, the aim of this study was to perform restriction enzymes of MC4R gene sequences in Bligon goat using BioEdit program.

MATERIALS AND METHODS

Blood sampling and DNA Isolation. The blood samples were collected from 40 ewe Bligon goats in Gunung Kidul, Yogyakarta. The blood is collected using vacutainer containing K3EDTA about 3 ml for each goat through jugular veins. Samples were isolated using *SYNC^{TM} DNA Extraction Kit* (Geneaid, Taiwan).

Primer design and amplification DNA. The MC4R gene target was amplified by PCR *(Polymerase Chain Reaction)* method using one set of primers F: 5'-TCGGGCGTCTTGTTCATCAT'-3 and R: 5'-CAAGACTGGGCACTGCTTCA'-3. The size of PCR target was 642 bp. The target is located in base 924-1385 (exon 1) and base 1386-1565 (3'UTR). (reference sequence Genbank accession no. NM_001285591). PCR was performed in the 30 ml total reaction volume, comprising 10 µl of DDW, 1.5 µl of primer F and R, 15 µl of PCR kit (KAPPABIOSYSTEMS) and 2µl of DNA genome. DNA amplification was done by 35 cycles with the pre-denaturation temperature at 94^0C for 3 minutes, denaturation at 94^0C for 30 seconds, annealing at 59.7^oC for 30 seconds, extension at 72^0C for 30 seconds and followed by a final extension at 72^0C for 10 minutes.

Sequencing. The 30 µl of PCR products were sequenced using the same primer (forward primer) for PCR target (location in exon 1 and 3'UTR, n=10). Sequencing was performed by PT. Genetika Science, Indonesia.

Restriction enzymes analysis of MC4R gene sequences. The methods of identification of single nucleotide polymorphism, amino acid change, and restriction enzyme mapping were described by Hartatik (2016). The sequences result from sequence analysis of Bligon goat samples (n=10 samples) then aligned using Bioedit software (ver 7.2.0) to identify the single nucleotide polymorphism and restriction enzyme mapping.

RESULTS AND DISCUSSION

There were two SNPs (g.998A/G and g.1079C/T) successfully identified using DNA sequencing method. The SNP g.998A/G formed GG and GA genotypes, the SNP at g.1079C/T produce two genotypes (CC and CT), thus none of them have AA and or TT genotypes. Based on restriction enzyme mapping using Bioedit ver. 7.2.0 software, there were 6 enzymes which recognized 5 SNPs region, consisting of 3 enzymes 4-bases (*FatI, NlaIII* and *RsaI*) and 2 enzymes 6-bases (*Acc651* and *KpnI*). There were 3 enzymes that produced large fragments (>100 bp) and had a specific character. Among 5 enzymes, there were 3 recommended enzymes for genotyping the Bligon goat. The three recommended restriction enzymes cleavage the PCR product at the SNP g.1079 C/T. For C allele or base, *Rsa*I restriction enzyme recognized one site at 521 bp but not for other enzymes (*Acc*651 and *Kpn*I). For T allele or base, the *Rsa*I recognized two sites (157 and 521 bp) and others only recognized one site at 155 bp (*Acc*651) and 159 bp (*Kpn*I) (Figure 1).

In our study, three restriction endonucleases (*Rsa*I, *Acc*651 and *Kpn*I) may be used for genotyping Bligon goat using PCR-RFLP method. The *Rsa*I, *Acc*651 and *Kpn*I were recognized GT'AC, G'GTAC_C and G_GTAC'C; respectively. Previous research used restriction enzyme for genotyping ruminant was based on MC4R gene (Liu *et al.,* 2010; Wang *et al.,* 2015). Wang *et al.* (2015) reported that restriction enzyme *Msp*I, *Kpn*2I and *Nde*I were used for genotyping sheep. In another study, enzyme *Tai*I was used for PCR-RFLP and produced three genotypes in a Qinchuan cattle (Liu *et al.,* 2010). In non-ruminant, the enzyme *Taq1* produced 156 bp and 70 bp fragment in pig with allele 1, and allele 2 produced a 226 bp fragment as the PCR-RFLP (Kim *et al*., 2000). Three endonucleases were used

(*Tai1, Bs1* and *Sma1*) for genotyping dog and fox (Skorczyk *et al*., 2007). Regarding of report Zhou *et al*. (2012), the digest with *Fbr1* enzyme was detected three genotypes in chicken (C1C1, C1C2 and C2C2).

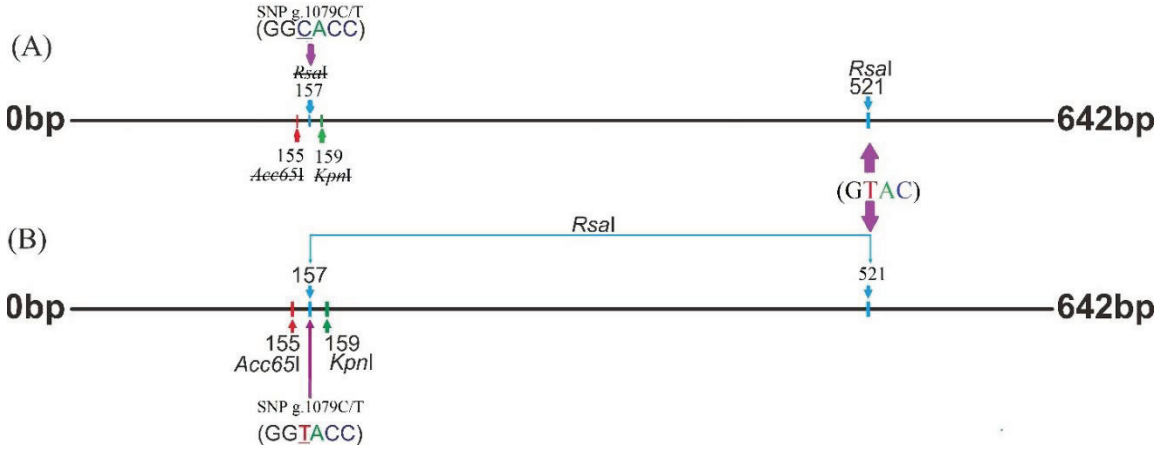

Figure 1. Illustration of 3 recommended restriction enzymes at SNP g.1079 C/T.

CONCLUSIONS

In conclusion, the restriction enzymes *RsaI, Acc651* and *Kpn1* may be used for genotyping of the targeted gene (MC4R) using PCR-RFLP method for genotyping of Bligon goat in the future research.

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