

IDENTIFICATION OF UTERIN MILK PROTEIN (UTMT) GENE IN BALI CATTLE USING DIRECT SEQUENCING

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ABSTRAK

Tujuan dari penelitian ini adalah untuk mengidentifikasi keragaman haplotipe fragmen gen UTMP ekson 5 pada sapi Bali menggunakan metode *direct-sequencing*. Sebanyak 60 sampel darah sapi Bali yang berasal dari BPTU sapi Bali di pulau Bali (20 ekor), BPTU Serading di pulau Sumbawa (20 ekor) dan *Village Breeding Center* (VBC) di Kabupaten Barru Sulawesi Selatan (20 ekor) digunakan untuk mengevaluasi keragaman genetik gen UTMP pada ekson 5. Data hasil sekuensing *forward* dan *reverse* dianalisis menggunakan Bioedit dan disejajarkan menggunakan program MEGA5, sedangkan analisis haplotipe dilakukan menggunakan program DnaSPv5. Hasil dari sekuen fragmen gen UTMP ekson 5 menunjukkan ada 16 haplotipe dengan jumlah haplotipe terbesar ditemukan pada VBC Barru Sulawesi Selatan (8 haplotipe). Rataan keragaman haplotipe (h) dan nukleotida (p) tertinggi ditemukan di VBC Barru Sulawesi Selatan sebesar 0.7949 dan 0.0016. Selain itu, ditemukan juga insersi berupa minisatelit dengan susunan sekuen 5'-CCA GTC ATG AAG AAG GCA GAG GTC GTC GTG CCG GCG AAA-3'. Berdasarkan hasil yang diperoleh, variasi haplotipe dan minisatelit pada fragmen gen UTMP ekson 5 dapat dijadikan sebagai kandidat marka genetik spesifik sifat reproduksi pada sapi Bali dan strategi pemuliaannya di masa yang akan datang.

Kata kunci: gen UTMP, haplotipe, keragaman, sapi Bali

ABSTRACT

The objective of this research was to identify diversity of exon 5 UTMP gene fragment in Bali cattle using direct sequencing. The total 60 blood samples of Bali Cattle derived from BPTU Bali in Bali island (20 heads), BPTU Serading in Sumbawa island (20 heads) and Village Breeding Center in Barru District South Sulawesi (20 heads) were used to evaluate their genetic diversity at exon 5 UTMP gene. The forward and reverse data sequences were analyzed using Bioedit program and alignment analysis was carried out using MEGA5 program. Meanwhile haplotype analysis was performed by DnaSPv5 program. The result showed that partial sequences in exon 5 UTMP gene had 16 haplotypes with the highest number of haplotypes were found in VBC Barru district South Sulawesi (8 haplotypes). Moreover, the highest average of haplotype (h) and nucleotide (p) diversity were found in VBC Barru district South Sulawesi were 0.7949 and 0.0016, respectively. In addition, minisatellite insertion was found in exon 5 UTMP gene fragment on Bali cattle which are consist of 5'-CCA GTC ATG AAG AAG

GCA GAG GTC GTC GTG CCG GCG AAA-3'. According to our results, haplotype and minisatellite variation in exon 5 UTMP gene fragment can be used as a candidate genetic marker specific for reproductive trait in the Bali cattle and for its strategy breeding program in the future.

Keywords: UTMP gene, diversity, haplotype, Bali cattle

INTRODUCTION

The uterine milk proteins (UTMP) gene known as serine proteinase inhibitor member of 14 (Serpina 14) (Kandasamy *et al.*, 2010), is a protein secreted by the endometrial epithelium during pregnancy (Padua and Hansen, 2010). Serpina 14 (UTMP) gene has been further implicated as one of the important candidate genes for embryonic survival in the bovine (Khatib *et al.*, 2007). Moreover Khatib *et al.*, (2007) showed that UTMP gene expression found predominantly in reproductive tissues and play an important role in reproductive traits and it was located on chromosome 21, which consists of five exons (Ulbrich *et al.*, 2009). It was further reported, the diversity of UTMP genes significantly affect fertilization and embryo survival rates in dairy cows (Khatib *et al.*, 2009).

An Indonesia native animal genetic resources, Bali cattle has advantages in reproductive trait (Purwantara *et al.*, 2012). It showed high reproductive capacity (high fertility and calf each year over a long time) (Entwistle and Lindsay 2003), higher reproductive efficiency than the cross-bred (Pribadi *et al.*, 2015) and high conception rate in different regions (Australia, Malaysia, Bali and Timor) (McCool 1992). Until now, the information of the genetic characteristics of Bali cattle especially UTMP gene as a potential gene for reproductive traits were still limited. Based on many studied, it was already known that growth hormone receptor (GHR) gene (Zulkharnaim *et al.*, 2010), growth hormone (GH) gene (Jakaria and Noor 2011), pituitary specific transcription factor-1 (Pit-1) gene (Jakaria and Noor 2015), calpastatin gene (CAST) (Putri *et al.*, 2015) and prolactin (PRL) and signal transducers and activators of transcription 5A (STAT5A) genes (Paramitasari *et al.*, 2015) had low genetic diversity using PCR-RFLP technique. Therefore, the aim of this study is to identify diversity based on haplotypes in exon 5 UTMP gene fragment in Bali cattle by direct sequencing technique. This study is important to designed the strategy of Bali cattle breeding program in the future.

MATERIAL AND METHODE

A total DNA sample of 60 blood samples of Bali cattle from BPTU Bali in Bali island (20 heads), BPTU Serading-Sumbawa NTB (20 heads) and Village Breeding Center (VBC) Barru South Sulawesi (20 heads) were extracted using DNA extraction kit (GeneAid protocol). Primer used to amplify the exon 5 UTMP gene fragment based Khatib *et al.*, (2007) was 5'- GGC CCT TCA ACA AGC TGA GA -3' (forward) and 5'-CTA GGG CTC TTG AAC GTT GA -3' (reverse) with length of PCR product 289 bp (Figure 1).

Amplification of exon 5 UTMP gene fragments used thermo cycler AB system machine added by PCR reagent (50 µL) containing 10 µL buffer 10x, 2 µL MgCl₂, 0.5 µL of dNTP, 0.5 µL of forward primer and 0.5 µL of reverse primer, 0.2 µL of Taq polymerase and distilled water 39.3 µL. PCR machine was performed with this following temperature condition: pre-denaturation at 95°C for 5 min, 35 cycles (denaturation at 95°C for 10 seconds, annealing at 60°C for 20 seconds, and extension 72°C for 30 seconds), post extensions at 72°C for 5 minutes. PCR products were electrophoresed using 1.5% agarose gel contained EtBr. Furthermore, PCR products were sequenced using the machine sequencer (ABI Prims 3100-Avant Genetic Analyzer) in the 1st Base sequencing company, Selangor, Malaysia. The forward and reverse of exon 5 UTMP gene fragment sequences were edited using Bioedit (Hall, 1999) and MEGA5 programs (Koichiro *et al.*, 2011). Haplotype analysis was carried out by DnaSP v5 program (Librado and Rozas, 2009).

RESULT AND DISCUSSTION

Amplification of Exon 5 UTMP Gene

Optimum temperature of exon 5 UTMP gene fragment in Bali cattle was obtained at 60°C for 20 seconds. Figure 2 showed that the PCR products of exon 5 UTMP gene fragment in Bali cattle (405 bp) were different from the PCR product reference (289bp with Genbank access number L22095). PCR product of Bali cattle was longer than the one of reference because there was

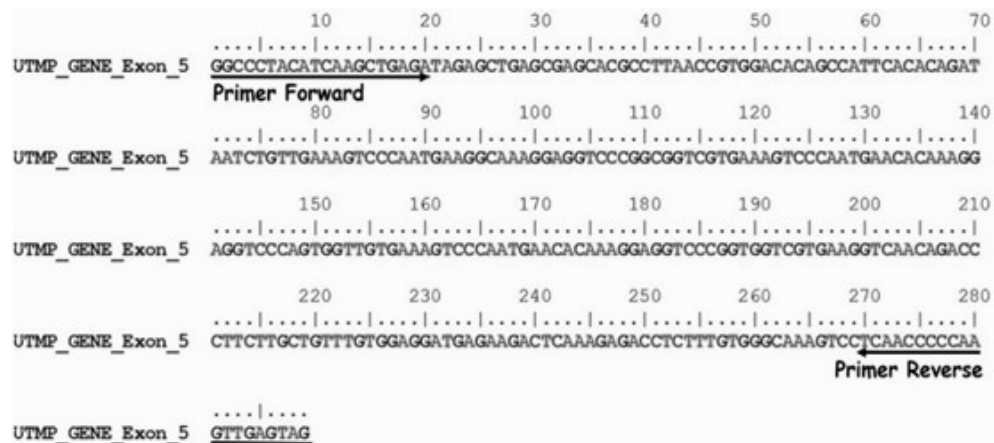


Figure 1. Amplification Primer (underline) in the Sequence of the Exon 5 UTMP Gene Fragment (GenBank access number L22095).

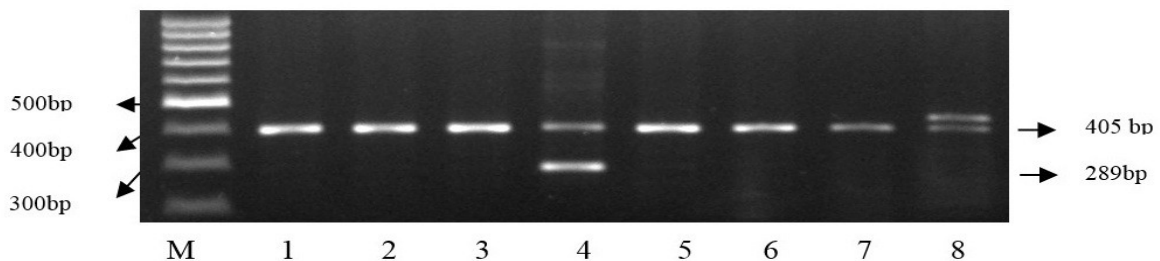


Figure 2. PCR Product Electrophoresis of Exon 5 UTMP Gene in Bali Cattle. M is marker of 100 bp, line 1-8 is sample of Bali cattle

an insertion mutations in Bali cattle PCR product. Li and Graur (1991) stated that the insertion is one type of mutation that increases one or more nucleotide in DNA sequence.

Nucleotide Diversity of Exon 5 UTMP Gene

Haplotype analysis identified 16 haplotypes in the exon 5 UTMP gene fragment from Bali cattle. They were distributed in BPTU Bali cattle in Bali Island (5 haplotypes), BPTU Bali cattle in Serading-Sumbawa NTB (5 haplotypes) and VBC Barru South Sulawesi (8 haplotypes) as presented in Figure 3. A total 16 haplotypes containing around 50 single nucleotide polymorphisms (SNPs) found in three Bali cattle populations may be used as a potential new candidates marker for reproductive traits in cattle Bali (Figure 3). The number of haplotypes (H), haplotype diversity (h)

and nucleotide diversity (π) of Bali cattle are presented in Table 1.

The highest number of haplotypes was found in the Bali cattle population from VBC Barru South Sulawesi. Similarly, the highest value of the haplotype (h) and nucleotide (π) diversities were detected in Bali cattle population in South Sulawesi VBC Barru, they were 0795 and 0016, respectively. Haplotype and nucleotide diversity of exon 5 UTMP gene which were found in Bali cattle population is very important. Goodall-Copestake *et al.*, (2012) stated that the genetic diversity is an important as a reference for the conservation and management programs.

Minisatellite motive insertions in exon 5 UTMP gene fragment with sequence nucleotide 5'- CCA GTC ATG AAG AAG GCA GAG GTC GTC GTG CCG GCG AAA -3 'was found from

Table 1. Exon 5 UTMP Gene Diversity in Three Populations of Bali Cattle

Bali Cattle Population	n	H	h	π
VBC Barru district South Sulawesi	20	8	0.795	0.016
BPTU in Bali island	20	5	0.585	0.005
BPTU in Sumbawa NTB	20	5	0.708	0.010

n: individual number, H : number of haplotype, h : haplotype diversity, π : nucleotide diversity

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[          10          20          30          40          50]
[          *          *          *          *          *]
Hap_1 ACAGAGCCGCGAGTCAAGAACGTACAATTACAGTGAAGCCCTCGACAAGGG
Hap_2 .....GGG.C...A.....
Hap_3 .....GGGGCC...A.....
Hap_4 .....T.....GGGG.C...A.....
Hap_5 .G.C.CATT.G.C.....A..C..
Hap_6 .G.C.CATT.G.C.....G...G.....T.....A..C..
Hap_7 .G...CATT.G.C.....G.....AC.CAA
Hap_8 .G...CATT.G.C.....G.....T.....AC.CAA
Hap_9 C.C...T.GGCCGGCCCGCG.GGGCC.GG.C.CCTAAGGTTGACTC.A
Hap_10 .....T.....GGGCC.GG.....
Hap_11 .....GGGCC.GG.....
Hap_12 .....GGGCC.GG.C.....
Hap_13 .....GGGCCCGG.....
Hap_14 .....T.....GGGCCCGG.....
Hap_15 .....GGGCCCGG.C.....
Hap_16 ...CT.ATTGG.CG....GG...GGGCC.GG.CAC.TA..G...A.T..A

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Figure 3. The Haplotype Diversity of Exon 5 UTMP Gene Fragment in Bali Cattle

sequencing analysis (Figure 4). Pathak *et al.*, (2010) reported that the classification of minisatellites were 10 up to 100 bp on the number of nucleotide sequences. It is clear that the length of PCR products difference between the this study (405 bp) the one of fragment reference (289 bp) were come from variations of minisatellite in exon 5 UTMP gene. Insertion minisatellite in exon 5 UTMP genes may influence the expression of the produced protein and the possible effect on the gene function. Amino acid sequences generating minisatellite insertion are VPMKAKEVPAVVK. The uniqueness of minisatellite insertion sequences in the exon 5 UTMP gene fragment was only found in Bali cattle population and it was not found in GenBank reference sequences such as reference sequences in UTMPBT201006 Simmental, BT301003 Limousin and L22095 access numbers. The diversity of minisatellite marker could be used as

a genetic marker in the study of functional and comparative genomics analysis (Pathak *et al.*, 2010).

Genetic diversity of exon 5 UTMP gene utilization has been reported by Khatib *et al.*, (2007), namely mutation c1.779A>G and c1.296A>G associated with the increase of the lifetime of productive FH cattle. In addition, the GG genotype has lower embryos survival than GA and AA genotypes. Kandasamy *et al.*, (2010) stated that gene expression UTMP was controlled by progesterone synthesis and predominantly found in the endometrium playing a role in maintaining pregnancy. UTMP gene expression also plays as important factor in the productive living age of dairy cattle (Khatib *et al.*, 2009). Genetic diversity particularly haplotype diversity and minisatellite obtained in exon 5 UTMP gene in Bali cattle can be used as a marker for reproduction marker candidate. However, it is

needed to investigate the association between haplotype and reproductive trait. Therefore, the haplotype diversity and minisatellite can be used as a reference in the Bali cattle breeding program strategy in the future.

CONCLUSION

Analysis of genetic diversity of exon 5 UTMP gene on Bali cattle found 16 haplotypes and highest haplotype and nucleotide diversity were detected in VBC Barru district South Sulawesi. This study also identified minisatellite motive sequences in exon 5 UTMP gene of Bali cattle. Genetic diversity in exon 5 UTMP gene fragment can be used as a candidate genetic marker specific in the Bali cattle and for breeding program strategy in the future.

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