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THE SRY GENE VARIATIONS AMONGST SELECTED MADURA CATTLE POPULATIONS

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ABSTRACT

The aim of this study was to determine the diversity of the sex-determining region Y (SRY) gene in Madura cattle bulls that had specifically selected for the production of frozen semen. DNA was isolated from the whole blood derived from 5 Madura cattle bulls. The SRY gene amplification was carried out using polymerase chain reaction (PCR) with SRY-4 as a primer followed by DNA sequencing and BLAST analysis to find out nucleotid variation and to construct filogenetic tree between Madura cattle bulls. Results revealed the proximity of the Madura cattle SRY genes to the *Bos indicus* SRY genes, furthermore this study also proved the existence of variation in Madura cattle SRY gene caused by mutation and deletion of nucleotides. It was concluded that the variations in Madura cattle SRY genes still persist even in the specifically selected populations with similar phenotype.

Key words: Madura cattle, SRY variation, sequence analysis

ABSTRAK

Tujuan penelitian ini adalah mengetahui keragaman gen SRY pada sapi Madura jantan yang telah diseleksi untuk produksi semen beku. Amplifikasi gen SRY dilakukan melalui PCR dengan primer SRY-4 dari isolat DNA yang berasal dari darah utuh 5 ekor sapi Madura jantan yang dilanjutkan dengan pengurutan DNA dan analisis menggunakan BLAST untuk mengetahui variasi nukleotid dan membangun pohon filogenetik antar sapi Madura jantan. Hasil penelitian menunjukkan kedekatan gen SRY sapi Madura terhadap gen SRY Bos indicus dan terdapat keragaman pada gen SRY antar sapi Madura berupa mutasi dan delesi nukleotida walaupun dalam jumlah yang rendah. Penelitian ini menunjukkan keragaman pada gen SRY masih bisa terjadi pada sapi Madura meskipun telah diseleksi secara khusus dalam aspek fenotip yang sama.

Kata kunci: sapi madura, keragaman SRY, analisis sekuensi

INTRODUCTION

Sex-determining region Y (SRY) gene has been known as testes genes and male sex determination factor. It was haplotypes and no recombination occurs during meiosis made it conserved and only inherited by the male lineage (Liu and Ponce de Leon, 2007). Moreover, the SRY gene diversity was reported low due to the slow rate of mutation (Mburu and Hanotte, 2005). Although genetic diversity of SRY gene was fairly low, SRY gene has a high enough diversity between species, has made it widely used to determine the origin of modern livestock from the sire lineage based on its proximity between species (Syed-Shabtar *et al.*, 2013).

The diversity of genes, including the SRY gene would be exists in a natural population. Previous study has shown variations of SRY gene in one of Indonesian indigenous cattle species actually exist (Winaya *et al*., 2012). Similar research might be useful to observe the SRY gene variation in another indigenous cattle especially Madura breed that have been selected for frozen semen productions. Therefore, the aim of this research was to study the diversity of the SRY gene on the Madura cattle stud which has been selected for the frozen semen productions.

MATERIALS AND METHODS

The animals used in this study were 5 Madura cattle bulls that have been selected for frozen semen production in artificial insemination center in Singosari, Indonesia. The five bulls used in this study have passed the bull soundness examinations and have declared eligible as a stud and produce normally.

DNA Preparation

The DNA was isolated from the whole blood of five Madura cattle bulls by chloroform: Isoamyl-alcohol methods described by Sambrook *et al.* (1989). DNA amplification was carried out by PCR with primers of SRY-4: 5 '- GCC TGG ACT TTC TGC TTG TTA - 3' and reverse 5 '- ACA GTG GGA GAC AAA ACA TAT - 3' for *Bos javanicus* (Verkaar *et al*., 2003). All the stages from the DNA isolation to PCR was performed at Laboratory of Molecular Biology, University of Brawijaya while DNA sequencing was performed by Apical Scientific Sdn. Bhd.

Sequence Analysis

The NCBI-BLAST (http://blast.ncbi.nlm.nih.gov/ Blast.cgi#) sequence analysis was performed to compare sequences between bulls and species comparison by the alignment and build a phylogenetic tree.

RESULTS AND DISCUSSION

Y chromosome genes in male-specific region of the Y chromosome (MSY) did not recombine during meiosis, therefore the diversity within a species were relatively very small due to the low rate of mutations in

Y chromosome genes, including SRY (Mburu and Hanotte, 2005; Nijman *et al.*, 2008). Despite a low diversity within species, the SRY gene has a high diversity between species. For that reason, the SRY was widely used to determine an individual's genetic proximity into species level (Syed-Shabtar *et al.*, 2013).

Madura cattle breeds was believed a croosbreed of two species of *Bos indicus* and *Bos javanicus* although phenotypically closer to the *Bos indicus* characterized by hump owned (Payne and Rollinson, 1976). Paternal lineage of Madura cattle was believed to have originated from *Bos indicus* (Nijman *et al*., 2003; Mohamad *et al*., 2009) while the maternal lineage originated from *Bos javanicus* (Kikkawa *et al*., 2003) although Verkaar *et al*. (2003) also reported that Bos javanicus Y chromosome has found in some Madura cattle bulls.

SRY gene was always being inherited through the paternal lineage; therefore, it can be used to trace the origins of a species from its proximity. The SRY-4 amplification in our study produced the 580 bp of Madura cattle bull SRY sequences. The BLAST phylogenetic analysis showed that the SRY gene of our Madura cattle bull were close to the *Bos indicus* SRY gene rather than *Bos javanicus* (Figure 1), despite of the primers used for DNA amplification derived from *Bos javanicus* SRY. On the subject of paternal lineage, these results were similar to those reported previously by Nijman *et al.* (2003) and Mohamad *et al.* (2009). This study indicated that the Madura cattle bull in our study has a paternal lineage derived from *Bos indicus*.

In spite of Madura bull SRY gene had a proximity to Bos indicus DQ 336527.2, the sequence alignment showed the difference in the base number 1815 ~ 1817; 2372; and 2389 between Madura bull and Bos indicus DQ336527.2. The diversity of SRY gene was found in the form of A-C mutations in 1815 bp; C-T at 2372 bp; and G-A at 2389 bp that known as a SNP. Beside the mutations, the deletion also found in 1816 ~ 1817 bp of SRY Madura cattle bulls compared to the reference SRY (Figure 2).

The diversity of the SRY gene can be occurred due to mutations, alterations and micro deletion (Mukherjee *et al.*, 2013). The sequences analysis showed the deletion and mutation were exist in Madura bull SRY gene, indicating the diversity of SRY gene within Madura bulls (Figure 3). Based on our results, adenine deletion was found in 31 bp of bull number-30 while

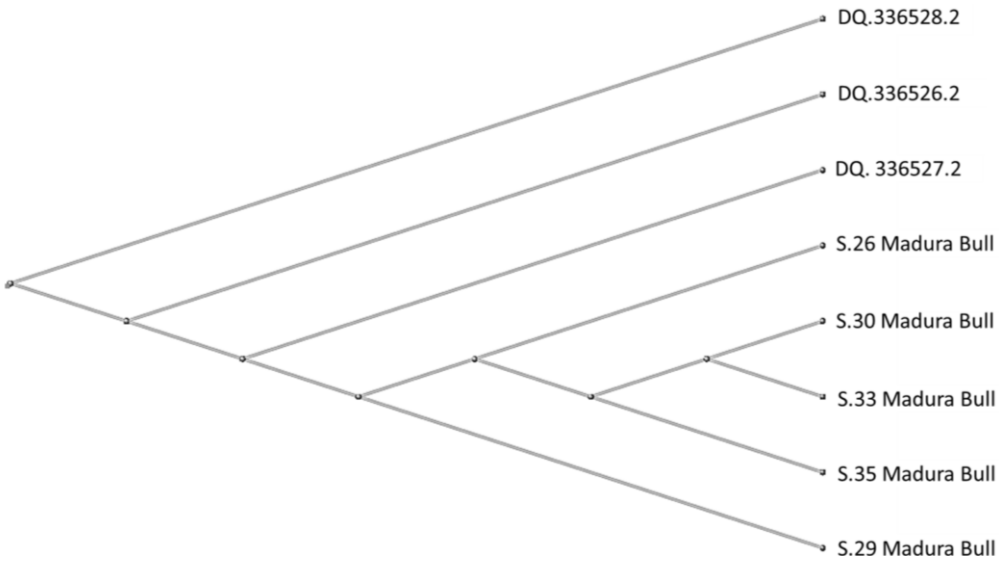

Figure 1. The phylogenetic tree based on the SRY gene of 5 Madura bull, *Bos javanicus*, *Bos indicus* and *Bos taurus* by the Fast Minimum Evolution Method Slanted Models. DQ 336527.2= *Bos indicus*, DQ 336528.2= *Bos javanicus*, DQ 336526.2= *Bos Taurus*, S.26, 29, 30, 33, and 35= Madura bulls

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                          1810       1820       1830       1840       1850       1860       1870       1880
                          .|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ...
DQ336527.2| Bos indicus   TACTCTC GCTAACAAAG GCGCTCTTTA TCTCAATTTT ACTACAGTTT CACCTGCGAC TTAATTTTAT AAGAAGCCCA ATA
s26                       TACTCTC GCTAC--AAG GCGCTCTTTA TCTCAATTTT ACTACAGTTT CACCTGCGAC TTAATTTTAT AAGAAGCCCA ATA
s29                       TACTCTC GCTAC--AAG GCGCTCTTTA TCTCAATTTT ACTACAGTTT CACCTGCGAC TTAATTTTAT AAGAAGCCCA ATA
s30                       TACTCTC GCTAC--AAG GCGCTCTTTA TCTCA-TTTT ACTACAGTTT CACCTGCGAC TTAATTTTAT AAGAAGCCCA ATA
s33                       TACTCTC GCTAC--AAG GCGCTCTTTA TCTCAATTTT ACTACAGTTT CACCTGCGAC TTAATTTTAT AAGAAGCCCA ATA
s35                       TACTCTC GCTAC--AAG GCGCTCTTTA TCTCAATTTT ACTACAGTTT CACCTGCGAC TTAATTTTAT AAGAAGCCCA ATA

                          2370       2380       2390
                          .|....| ....|....| ....|....| ..
DQ336527.2| Bos indicus   GTTTATA GCCTTTTGTT CCCACTGTGA AA
s26                       GTTTATA GTCTTTTGTC CCCACTGTAA AA
s29                       GTTTATA GTCTTTTGTT CCCACTGTAA AA
s30                       GTTTATA GTCTTTTGTT CCCACTGTAA AA
s33                       GTTTATA GTCTTTTGTT CCCACTGTAA AA
s35                       GTTTATA GTCTTTTGTT CCCACTGTAA AA
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Figure 2. SRY Madura bulls compared to reference SRY DQ 336527.2

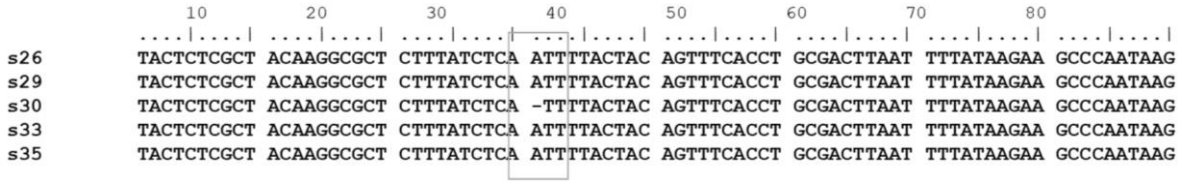

Figure 3. Variation of SRY gene amongst Madura bull

the T-C mutation was found in 575 bp of bull number-26. The rest, the bull number-29 has the uniform sequences to the bull number-33 and 35.

SRY gene was known as testis forming genes that control the male sex differentiation (Li *et al*., 2014). The diversity of the SRY gene due to mutation and deletion were associated to infertility and genital developmental disorders symptoms (DSD syndrome) in the form of hermaphroditism (Lu *et al.*, 2013). However, in this study the mutations and deletion could be found also in the SRY gene of stud with normal semen production. Nonetheless, it is very important to learn more about the diversity that found in the SRY gene of Madura cattle.

CONCLUSION

The proximity of the Madura cattle SRY gene to the *Bos indicus* SRY gene has shown the patrilineal relationship of zebu to Madura cattle. The specific selection for the frozen semen productions purposes was made the phenotypic uniformity amongst the bulls inside AI stations, nevertheless the diversity still occurred in the molecular levels that shown by the deletion and mutation in the SRY gene. Moreover, the diversity of SRY gene can lead the phylogenetic study to reveal the patrilineal ancestor of the modern Madura cattle.

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