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SRY Gene Marker Differences in Native and Crossbreed Cattle

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ABSTRACT

This study focused on the promoter region of the SRY gene with 1,281 bp DNA fragments, including 5'UTR, CAAT signal, and TATA box. Genomic samples of 19 cattle were obtained from Wagyu-BX (n = 2), BX (n = 5), Simmental (n = 2), Limousin (n = 2), Ongole (n = 2), Madura (n = 2), Bali (n = 2), Nellore (n = 1), and Hereford (n = 1). Two flanking primers (forward and reverse) were used for polymerase chain reaction (PCR). The PCR products were then sequenced by using a two-way primer. The obtained sequences were aligned with clustalW software to determine the differences in the nucleotide base arrangement which compiled the promoter region of the SRY gene. The cattle crossbreeding was done as an effort to improve the genetic variations and qualities. The SRY gene is a marker gene inherited from the male side (bull), so the SRY gene is expected to be used as a marker to monitor the crossbreeding. The monitoring of the crossbreed cattle is an initial effort to increase the genetic variations and enhance the genetic qualities without threatening the germplasm purity. The results of this study showed that the overall sample is monomorphic, except for Bali and Nellore cattle. Further research is needed by expanding the analysis area of the SRY gene and increasing the number of samples.

Keywords: Genetics marker, PCR, Promoter region, Sequencing, Sex-determining region Y (SRY)

Introduction

The existence of Y chromosomes determines typically the sex in mammals. Male sex would be determined by the normal function of the Y chromosome gene or Sex-determining region Y (Ruvinsky, 2015). Sex chromosomes (X and Y on mammals) are widely discussed as proof of genetic evolution (Ellegren, 2011). It could be seen from the similarity on the promoter of the SRY gene from different animal species, like in goat and cattle (Cheng *et al*., 2001). The similarity could also be seen from species in the different family, thus indicates a similar sex-determining gene function for the male sex determinant (Coriat *et al*., 1993). The sex chromosome could be originated from the autosome (Ellegren, 2011), with the X chromosome formed earlier then differentiated into Y chromosome (Chang *et al*., 2001; Ellegren, 2011). The theory is also supported by the finding of the SRY gene from SRY-box family, which is the SOX3. Furthermore, the SOX gene is currently found whether in vertebrate or invertebrate (Cheng *et al*., 2001).

A cattle embryo with XY chromosome (male) would show a faster gonad differentiation, at 25 – 27 days of pregnancy, while for a female the differentiation occurred several days later (Juarez-Oropeza *et al.,* 1995). The unexpressed SRY gene then stimulates the ovary development (Ross *et al.,* 2008). Most researches have been done on the High mobility group (HMG) box of SRY, a protein which acts as a transcription factor that binds and bends the DNA strands (Harley *et al.,* 1992; Coriat *et al.,* 1993). From the in vitro analysis, it can be seen that the HMG box has the ability to bind specific DNA sequence (AACAAT) and could bend the DNA up to 90^{o} (King dan Weiss, 1993).

The 5'-end and 3'-end of UTR are involved in the SRY gene expression. The TATA box is the main part which composed the 5'-end of UTR (Barrett *et al.,* 2013). Cheng *et al.* (2001) added

that the most important part of 5' UTR regarding the SRY gene regulation are CAAT box, TATA box, SRY-binding site, and Sp1-binding site. Research by Alam *et al.* (2012) and Han *et al.* (2010) showed that the existence of SNP on the promoter region which includes 5' UTR and TATA box indicates a different transcription activity between the genotypes formed by the SNPs.

SRY gene polymorphism on the coding region has already been identified on Madura cattle (Hartatik *et al.,* 2014), Friesian Holstein cattle, Sahiwal cattle (Mukhopadhyay *et al.,* 2011), Bali cattle (Hassanin dan Ropiquet, 2007), and buffalo (Zhang *et al.,* 2006). The polymorphism showed specific alleles type which can be used as an effective genetic marker to detect crossbreeding between livestock (Zhang *et al.,* 2006), based on the inherited mutation (polymorphism) similarity (Verkaar *et al.,* 2004). The promoter region of the SRY gene on native cattle or its crossbreeds has never been studied before. Thus this study becomes important to further understand the promoter region of the SRY gene of the breeds.

Materials and Methods

Cattles

Nineteen cattle from various breeds were used in this study. The observed breeds in this study are as follows: Wagyu x BX crossbreed (n= 2), BX (n= 5), Simmental (n=2), Limousin (n= 2), Ongole crossbreed (PO) (n= 2), Madura (n= 2), and Bali (n= 2). Wagyu breed act as the bull in the Wagyu x BX crossbreed. Other materials used in this study were two SRY gene sequence (GenBank) from Hereford breed (*Bos taurus;* Acc. No. AC232880.1) and Nellore breed (*Bos indicus;* Acc. No. NC_032680.1).

Blood samples and DNA isolation

DNA samples were isolated from blood, which were taken through the jugular or caudal vein. The blood samples were collected using a vacuum tube (Venoject, DB Indonesia) which contained K3EDTA anti-coagulant. DNA isolation was done in the Laboratory of Animal Breeding and Genetics, Faculty of Animal Science, Universitas Gadjah Mada by using DNA Extraction kit (Genaid, Taiwan).

DNA amplification (PCR)

Primers (Table 1) were aligned based on two reference sequences (Acc. No. AC232880.1 dan NC_032680.1) downloaded from Genbank database (www.ncbi.nln.nih.gov). Primer pairs (forward 5'-GGA TTG ACA CAT TTG GCT GA-'3, and reverse 5'-TTC TTA CCA CAG ACT GAC TTA GTGC-'3) flank the target gene at 1,281 bp (Figure 1).

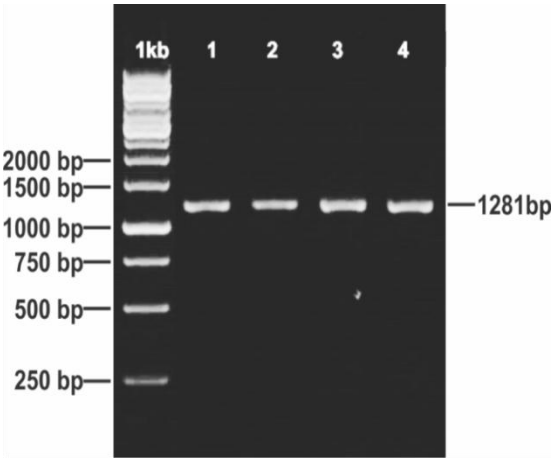

Figure 1. Visualisation of PCR product on the promoter region of Bali (lane 1-2) and Madura (lane 3-4) cattle SRY gene.

DNA sequencing and data analysis

PCR products as much as 30 µl/sample and 5 µl/sample of primers were sent to PT. Genetika Science Indonesia for sequencing. The obtained sequences were then aligned by using Bioedit software (7.2.5 version) to identify the existence of polymorphisms. A phylogenetic test was done based on the SRY gene sequence (1,281 bp) by using Mega 5 software, and the construction was done by using Maximum likelihood tree (1000 Bootstrap repetitions).

Result and Discussion

Five SNPs were obtained from the promoter region of SRY gene sequences alignment of 19 cattle. Three SNPs revealed on Bali cattle were at -966 C/G, -907 T/deletion, and -402 C/T, located before CAAT box, TATA box, SRY-binding, and Sp1-binding region. Two SNPs revealed on Nellore cattle (Bos *indicus*; GenBank Acc. No. NC_032680.1) was at -140 G/A and -117 G/A (Table 2), located after CAAT box, TATA box, SRY-binding, and Sp1-binding region.

Fifteen other sequences showed similar results on its nucleotide compilers. Polymorphism was not located on the important region for transcription processes such as CAAT box, TATA box, SRY-binding, and Sp1-binding. This finding is in accordance with Cheng *et al*. (2001) who observed a different family of Bovidae and found the SNPs on the promoter region that did not regulate the transcription process.

Table 1. Primers position based on the *Bos taurus* sequence (GenBank Acc. No. AC_232880.1)

Primer	Sequence	Location	Primer size
Forward	5'-GGA TTG ACA CAT TTG GCT GA-3'	148,857 – 148,876	20 bp
Reverse	5'-TTC TTA CCA CAG ACT GAC TTA GTGC -3'	150,113 – 150,137	25 bp

PCR product size = 1,281 bp, annealing temperature = 54°C.

However, single nucleotide polymorphisms which were found outside the transcription regulators might have certain consequences to the genetic regulation levels, regarding that the function between promoters is interrelated (Li *et al*., 2013). The upstream DNA region which was already known to affect transcription process is CAAT box, as it is the location for polymerase enzyme to initiate the transcription process, while also acts as the binding site for transcription factors such as NF1 and SP 1 (Kadonaga, 2004; Karp, 2010). The TATA box region plays a more vital role so that it is considered as the core promoter. The TATA box region is the location of the preinitiation complex which consisted of RNA polymerase II, while also acts as the binding site for most transcription factors required for the gene in eucaryotes to transcript DNA sequences (Kadonaga, 2004; Karp, 2010; Kadonaga, 2012). The SRY-binding site also acts as sequence transcription activators on the promoter region (Matsuzawa-Watanabe *et al*., 2003), while some parts are rich of A-T base nucleotides (Harley *et al*., 1992). The other transcription regulator on the promoter region is the Sp1-binding site, which exists in almost all genes. The Sp1-binding site acts as the base for the transcription process, mediating between signal and target genes which responded in one biological stream process (Samson dan Wong, 2002). Five SNPs found on the promoter region of SRY gene could impact the gene expression normality as each was interrelated (Li *et al*., 2013), and the occurred interaction on each part might impact the overall gene expression.

The existence of SNP in the promoter region of the SRY gene has been known to affect the genetic expression of the male sex. It is initially believed that the expression would be only determined by the HMG-box activity. However, further researches showed that the non-HMG-box also plays a certain role on the male sex expression (Zhao and Koopman, 2012). It can be seen from the abnormality in the promoter region of SRY gene which could cause failure or disturbance on the male gonad development, thus causing sex-reversal or lower fertility (Taqliarini *et al*., 2005). It is strongly suggested that the abnormality on the non-HMG-box would disturb the DNA target binding affinity and caused unstable interaction between the proteins responsible for transcription (Zhao and Koopman, 2012). On the other hand, the disturbance could also cause the disturbance in the SRY gene, a transcription factor, which initiate the Sertoli cell differentiation on testicle (Chen *et al*., 2015), even though the exact interaction between promoter region as a transcription factor is yet to be found (Zhao *et al*., 2016).

Bos genus consists of six species, namely *Bos taurus*, *Bos indicus, Bos sondaicus/Bos javanicus, Bos grunniens, Bos frontalis, Bos souveli* (possibly extinct) (Wilson *et al.,* 2005). In Indonesia, there are three Bos species exist, which are *Bos taurus, Bos indicus,* and *Bos sondaicus*. A crossbreeding between those Bos species is known to yield fertile offspring (Sutarno dan Setyawan, 2016), which can be seen from the most cattle in with various phenotypes which have *Bos sondaicus* mtDNA is the Indonesia native cattle (Mohamad *et al*., 2009; Priyadi *et al*., 2017).

Phylogenetic test on 19 cattle was divided into four groups; *Bos indicus* (Nellore; Acc. No. NC_032680.1), *Bos taurus* (Hereford; Acc. No. AC232880.1), 14 samples (homogenous samples), and *Bos sondaicus. Ovis aries* (AF026566.1) was used as an outgroup species in reconstructing the phylogenetic tree. The result of the phylogenetic tree reconstruction (Figure 2) showed that *Bos sondaicus* reside on different branch compared to the other three groups.

Bos indicus (Nellore; Acc. No. NC_032680.1) and *Bos taurus* (Hereford; Acc. No. AC232880.1) showed 0 likelihood based on the Bootstrap resampling. The furthest was shown by *Bos sondaicus* and the other 14 samples, with 0.003 likelihood. The genetical likelihood between 14 samples to *Bos indicus* group *Bos indicus* (Nellore; Acc. No. NC_032680.1), and *Bos taurus* (Hereford; Acc. No. AC232880.1) was 0.001, and the three groups shared same elders with low reliable bootstrap value (51%).

Cattles which were included in the 14 samples group showed a homogeneity on the promoter region of the SRY gene (1,281 bp). It can be said that the bulls of the group are *Bos taurus*, even though the occurred phenotype is currently tended to resemble *Bos indicus* (humped), in this case, were Madura and PO cattle. The similar situation also is shown in

Table 2. Nucleotide changes on the promoter region of SRY gene

Cattle breeds	N	Nucleotide changes				
		SNP1	SNP2	SNP3	SNP4	SNP5
Hereford (*Bos taurus*) (AC_232880.1)	1	C	T	C	G	A
Nelore (*Bos indicus*) (NC_032680.1)	1	N	N	C	R	R
Bali (*Bos sondaicus*)	2	G	Del	T	G	A
PO (*Bos indicus*)	2	C	T	C	G	A
Brahman Cross (BX) (*Bos taurus* x *Bos indicus*)	5	C	T	C	G	A
Wagyu (*Bos taurus*) x BX	2	C	T	C	G	A
Madura (*Bos indicus* x *Bos sondaicus*)	2	C	T	C	G	A
Limousin (*Bos taurus*)	2	C	T	C	G	A
Simmental (*Bos taurus*)	2	C	T	C	G	A

N: A/G/C/T, R = G/A.

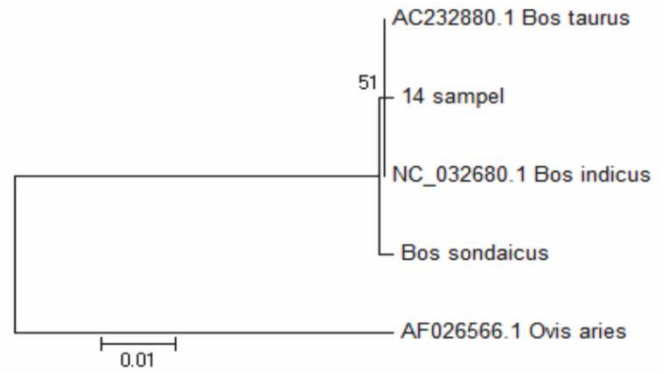

Figure 2. Phylogenetic tree based on the promoter region of the SRY gene (1,281 bp).

research done by Tanaka dan Namikawa (2002) on the Yunnan Chinese native cattle, where the occurred phenotype resembled *Bos taurus,* yet 96% of the population showed the SRY gene which resembles *Bos indicus*.

The study to determine SNP on the promoter region of Bali cattle SRY gene could be widely applied to check the cattle purity in Indonesia by tracing from the paternal line. Furthermore, it can also be used to support the breed purification program for Bali cattle as the Indonesia native cattle. Further research should be done with wider analysis area of the SRY gene, while also adding the research samples so that more comprehensive results can be obtained.

Conclusions

Five SNPs were found in the promoter region of the SRY gene. Bali cattle showed three SNPs (-966 C/G, -907 T/deletion, -402 C/T), while two SNPs were found in Nellore cattle (-140 G/A dan -117 G/A). Wagyu-BX, BX, Simmental, Limousin, PO, Madura, and Hereford cattle showed a uniform promoter region of the SRY gene.

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