Single Nucleotide Polymorphism of Sex Determining Region-Y Gene Coding Sequences in Belgian Blue Bull and Wagyu Bull Crossbred Cattle

T Hartatik1, S Bintara1, I Ismaya1, P Panjono1, B P Widyobroto1, A Agus1, I G S Budisatria1 and P Leroy2

1Faculty of Animal Science, UGM, Jl. Fauna No. 3 Bulaksumur, Yogyakarta 55281, Indonesia
2Faculty of Veterinary Medicine, University of Liege, Belgium
tety@ugm.ac.id

Abstract. Crossbreeding using exotic bulls have been developed widely in Indonesia. Y-chromosome markers have high value for the evaluation of paternal genetic and phylogeny history. The aim of this study was to investigate the coding sequence polymorphism of SRY gene in crossbred cattle using Belgian Blue Bull and Wagyu Bull. Polymerase chain reaction method was used to identify the target sequence 690 bp of SRY gene coding sequences. Then, direct DNA sequencing with forward primer was performed to identify the single nucleotide polymorphism (SNP) differences between individual sample. The results of the alignment coding sequence of SRY gene from four GenBank data, Brahman cross cattle, Belgian Blue Bull Cross and Wagyu Bull cross revealed that there are 21 SNPs variation. Six SNPs out of 21 SNPs (29%) was synonymous and 15 SNPs out of 21 SNPs (71%) was change the amino acid (non-synonymous). Eight out of 21 SNPs (38%) were transition mutation and 13 SNPs out of 21 SNPs (62%) were transversion mutation. Crossbred cattle using Brahman cross cow with Belgian Blue Bull and Wagyu Bull show the SNP at position 1707T→G (transversion mutation). This SNP change the amino acid from Phe(TTT) to Cys(TGT). Thus, the result can be used as molecular marker for identifying the paternal lineage of crossbred cattle which recently develop in Indonesia.

1. Introduction
The Brahman cross cattle performance can be improved by intensifying the genetic quality through crossbreeding with superior sire[1]. Wagyu and Belgia Blue are superior breeds which suitable for crossing with Brahman cross cattle. Wagyu has the superiority on meat marbling, so the meat had a great vogue[2]. Belgian Blue has high feed efficiency, high carcass percentage, and produce lean meat[3]. The combination of those two superior breeds will improve the meat quality and meat production of the crossbred beef cattle in the future.

Crossbreeding program can be controlled by genetic marker method. One of the genes that can be a genetic marker is the SRY gene. The Y chromosome-linked SRY gene is responsible for male sex determination in mammals. The SRY encodes a protein with a central HMG-box present in a wide
variety of proteins that bind and bend DNA, suggesting that SRY functions as a transcription factor [4]. In the previous study, the monitoring of the crossbred beef cattle is an initial effort to increase the genetic variations and enhance the genetic qualities without threatening the germplasm purity. The results of coding sequence (CDS) in SRY gene showed that the overall sample is monomorphic, except for Bali and Nellore cattle[5]. The study of Hartatik et al. [6] was identified 68.18% of Limousin x Madura crossbred cattle in the sequence of Y-chromosome. Also, the gene markers(SNP) of IGFBP3 were found at nucleotide base number 3,930 G/A in intron region in crossbreed cattle [7]. Therefore, the aim of this study was to investigate the coding sequence polymorphism of SRY gene in crossbred cattle using Belgian Blue Bull and Wagyu Bull

2. Material and methods

2.1. Collecting DNA sample
The blood samples were collected from 10 crossbred cattle (BX=4; Belgian Blue x BX = 4; Wagyu x BX=2). Then, the blood samples were isolated using GENEAID isolation kit (Taiwan)

2.2. Polymerase chain reaction
The target coding sequence 928 bp of SRY gene was amplified using forward primer GTTGATGGGTTTGGCGCTGACT and reverse primer AAATTGAATAAAGAGCGCCT with PCR kit 12.5 µl (My Taq HS Red 2x), double distilled water 9.5 µl and 2 µl DNA. The program consisted of an initial denaturation for 5 min at94°C followed by 35 cycles of 1 m at 92°C, 1 m at 60°C and 1 m at 72°C and by a final extension for 7 min at 72°C.

**Table 1. Location of Coding Sequence target base on GenBank Acc.No. AB039748.1**

| Target          | Sequence            | Size (bp) | Location | Amino Acid |
|-----------------|---------------------|-----------|----------|------------|
| Primer Forward  | gtgtatgggtttgggctgact | 21        | 771-891  |            |
| Primer Reverse  | aaattgagataaagagegct | 21        | 1778-1798|            |
| Start Codon     | ATG                 | 3         | 1067-1069| Methionine |
| Stop Codon      | TGA                 | 3         | 1754-1756| STOP       |
| CDS             | ATG….TGA           | 690       | 1067-1756| 230aa      |
| PCR Target      | GTT….TTT           | 928       | 771-1798 |            |

2.3. Sequencing
The PCR products as much as 25 µl/sample and 10 µl/sample of primers were sent to PT. Genetika Science Indonesia for sequencing. The PCR products were sequenced using sanger dideoxy sequencing method.

2.4. Alignment and single nucleotide polymorphism identification
Genbank accession codes for alignments are AB039748.1, AF148462.1, AY079145.2, AY341337.1, DQ119747.1. The alignment was formed for identification of single nucleotide polymorphism (SNP). The analysis of amino acid change used Bioedit program with choose graphic view in menu file.

3. Result and discussion
The SRY gene sequence of consist 928 bp with direct sequencing. Based on GenBank accession no. AB039748.1, the location of SRY gene in this study was 771-1798 bp. This part is covered the exon region of SRY gene as long as 690 bp. In the previous study was identified of polymorphism SRY gene in the promotor region on native and crossbred cattle[5].

In the Yak (Bos grummiens) and Chinese native bovine (Bos taurus) were characterized of SRY gene in the 3 region (5‘UTR, exon, and 3‘UTR). Three regions of the Bovidae SRY genes are aligned separately for comparison. In the 5’UTR region, the alignment shows a high level of sequence
conservation among these species except for four variable regions due to insertion/deletion (motif A-D). The sequences in the 3’UTR region are more conserved with the exception of 2 insertion/deletion of 4 bases. The alignment of the SRY exon regions from yak, Chinese native cattle and other species in Bovidae shows much higher amino-acid identities along the entire exon region[8]. The target of SRY gene of the crossbred cattle are aligned for comparison. Table 2 presents a comparison of the SRY gene in crossbred cattle and other GenBank that were registered in NCBI.

| Sample    | Single Nucleotide Polymorphism site |
|-----------|------------------------------------|
| AB039748.1| T C C G C G G C G A C A G C G C A C G |
| AF148462.1| T C C G C G G C G T C A G C G C C A C T |
| AY079145.2| T C C G C G G C G A C A G C G C C A C T |
| AY341337.1| G A T A A A T C T A G C T A A A T T G A T |
| DQ119747.1| G A T A A A T G T A G C T A A A T T G A T |
| Brahman Cross | T C C G C G G C G A C A G C G C C A C T |
| BBB Cross   | T C C G C G G C G A C A G C G C C A C G |
| WB Cross    | T C C G C G G C G A C A G C G C C A C G |

Six SNPs out of 21 SNPs (29%) was synonymous and 15 SNPs (71%) was change the amino acid (non-synonymous). Eight of 21 SNPs (38%) were transition mutation and 13 SNPs (62%) were transversion mutation (Table 3). Previous studies described the sequence variation in the Y-chromosomal SRY gene on crossbred cattle showed the contribution of Bos Taurus gene through the paternal line. There were 68.18% change of sequence type of Y Chromosome in crossbred cattle. The number showed that the consistent using of Limousin bull can continually shift the genetic trait from Y chromosome on local cattle[6]. The representative sample sequence has been submitted to the genbank with register ID BankIt2283096 and accession number MN727883 (Brahman cross, BX627), MN727884 (BBB634, Belgian Blue Bull cross) and MN727885 (WB507W, Wagyu Bull cross).

| No. | SNP | Mutation | Amino Acid Changes | Type         |
|-----|-----|----------|--------------------|--------------|
| 1   | SNP1(1105T→G) | Transversions | Ala(GCT) Ala(GCG) | Synonimous |
| 2   | SNP2(1122C→A) | Transversions | Thr(ACG) Asn(AAT) | Non-Synonimous |
| 3   | SNP3(1125C→T) | Transitions | Thr(ACG) Ile(ATT) | Non-Synonimous |
| 4   | SNP4(1183G→A) | Transitions | Gln(GAG) Gln(CAA) | Synonimous |
| 5   | SNP5(1199C→A) | Transversions | His(CAT) Asn(AAT) | Non-Synonimous |
| 6   | SNP6(1226G→A) | Transitions | Val(GTC) Ile(ATT) | Non-Synonimous |
| 7   | SNP7(1253G→T) | Transversions | Val(GTG) Leu(TTG) | Non-Synonimous |
| 8   | SNP8(1268C→G) | Transversions | Arg(CGA) Gly(GGA) | Non-Synonimous |
| 9   | SNP9(1280G→T) | Transversions | Val(GTG) Leu( TTG) | Non-Synonimous |
| 10  | SNP10(1292T→A) | Transversions | Asn(AAT) Tyr(TAT) | Non-Synonimous |
| 11  | SNP11(1315C→G) | Transversions | Asp(GAC) Glu(GAG) | Non-Synonimous |
| 12  | SNP12(1333A→C) | Transversions | Gly(GGA) Gly(GGC) | Synonimous |
| 13  | SNP13(1400G→T) | Transversions | Ala(GCC) Ser(TCC) | Non-Synonimous |
| 14  | SNP14(1409C→A) | Transversions | Arg(CGA) Arg(AGA) | Synonimous |
| 15  | SNP15(1449G→A) | Transversions | Arg(AGA) Lys(AAA) | Non-Synonimous |
| 16  | SNP16(1456G→A) | Transversions | Lys(AAG) Lys( AAA) | Synonimous |
| 17  | SNP17(1461C→T) | Transversions | Pro(CCA) Leu(CCT) | Non-Synonimous |
| 18  | SNP18(1564C→T) | Transversions | Tyr(TAC) Tyr(TAT) | Synonimous |
| SNP   | Transition | Amino Acid | Reference | Function       |
|-------|------------|------------|-----------|----------------|
| 19    | SNP19(1668A→G) | Transitions | Lys(AAG) | Arg(AGG) | Non-Synonimous |
| 20    | SNP20(1695C→A) | Transversions | Ala(GCG) | Glu(GAG) | Non-Synonimous |
| 21    | SNP21(1707G→T) | Transversions | Cys(TGT) | Phe(TTT) | Non-Synonimous |

4. Conclusion
Transversion mutation at SNP 1707T→G change the amino acid phe(TTT) to Cysteine(TGT). Therefore, the result can be used as molecular marker for identifying the paternal lineage of crossbred cattle which recently develop in Indonesia.

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