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Identification of Pure Breed Bali Cattle by Using Molecular Approach

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ABSTRACT: Bali cattle are well known as local beef cattle from Indonesia and recognized as one of beef cattle gene pools in South East Asia. The Bali cattle now are distributed almost throughout of Indonesia and most of them are in the status of either inbreeding or crossed breed. This recent research was intended to identify the pure breed of Bali cattle by using molecular marker approach. DNA Bali cattle samples collected from several locations in Indonesia were investigated. Amount of 24 DNA samples (23 males, 1 female) were used. Those 23 male DNAs consisted of male Bali cattle from NTB/West Nusa Tenggara (10), Riau (5), Bali (5), Kalimantan (2), and 1 male Brahman as breed control collected from Sembawa (Palembang) while one (1) DNA female was Bali cattle as sex control. An UTY gene was used in this study. All 24 DNA samples were amplified with UTY (F and R) primer for 35 cycles. Visualization of all PCR products on 1% agarose gel showed bands at 484 bp. as a right size for UTY gene, and none UTY fragment for female DNA sample (Bali cattle). Six UTY fragments were sequenced as representative of each region. The molecular analysis by ClustalW Alignment of the sequence results with reference of Genbank-NCBI showed that there was not found nucleotide different between sequenced samples to UTY reference, however there found 16 nucleotide different of sequenced samples to UTY gene reference. Similarity of UTY sequence was found 100% for sequence samples to UTY Bos Taurus and Bos indicus. This study concluded that UTY gen exists in all male Bali and male Brahman cattle. This early finding suggests that purity identification of Bali cattle needs more specific genetic marker in the Y-chromosome.

Keywords: Pure breed, Bali cattle, molecular analysis, UTY gene

INTRODUCTION

Bali cattle is one of local beef cattle breed in Indonesia and one of the four existing indigenous cattle breeds (Aceh, Pesisir, Madura and Bali) and Ongole breed was also considered as a local beef cattle since already adapted in a very long time in Indonesia (Martojo, 2012). The Bali cattle is well known as one of gene pools of beef cattle breed in Southeast Asia. In the past of a colonial era, distribution of Bali cattle was restricted only in Bali island in order to keep their breed purity. Up to now, however the Bali cattle almost distributes throughout of Indonesia and suspected in the status of not pure breed anymore.

Characteristics of Bali cattle are signed specifically with white rump patches and white stocking of four beneath legs, black hairs of the back, black color in male and brown in female and in juvenile. In the field, out site of Bali island, Bali cattle is sometime observed with white spotted and even almost all white color. It seems that inbreeding and crossing with other breeds were happened among Bali population. As consequence of inbreeding among Bali cattle, weight performance of Bali cattle now decreases as reported by Talib *et al.* (2000). A higher rate of inbreeding of Bali cattle might be happened in Bali since Bali cattle has been conserved for decades during colonial era. Purity of Bali cattle is believed conserved at Nusa Penida of Bali island. However, the average of their body weight performance is recently smaller (± 300-350 kg) compared to during colonial era (700-800kg).

There are some concerns about the breed purity because of intensive crossbreeding programs using natural mating and AI using exotic breeds that may cause extinction because of

indiscriminate crossing (Martojo, 2012). Conservation of Bali cattle purity needs to be prevented in order to develop breeding programs. Breeding of Bali cattle as well as other domestic local cattle in Indonesia is important to support a sustainable livestock production. In addition, Bali cattle has some advantageous of adapted at arid area, easy to be reared by smallholder farmers, better in reproduction, carcass and meat quality traits.

Recent molecular studies have reported the advantageous of applying molecular technology approaches. Applying of Single Nucleotide Polymorphism (SNP) in the intron of Y-chromosomal gene UTY 19 was used for cattle domestication (Götherström, *et al*., 2005; Svensson and Götherström, 2008). Genetic markers from Y-chromosome have been used for tracing the origin of Bali cattle (Kusdiantoro *et al.,* 2009). Purity assessment of Bali cattle has been conducted by applying specific microsatellite marker of HEL9 and INRA035 (Handiwirawan *et al*., 2003). Breeding value estimation and genetic tendency can also be used for evaluation of Bali cattle purity (Sukmawati *et al*., 2002). Genotyping by applying microsatellite genetic markers can be used for parentage checking (Margawati *et al*., 2002).

This research was therefore designed to apply molecular marker to define the purity of Bali cattle. As early study, a genetic primer of UTY was applied for this research purpose in male Bali cattle from several locations in Indonesia and female Bali cattle as sex control and Brahman cattle as breed control.

MATERIALS AND METHODS

Samples and DNA Isolation. Amount of 24 sample animals was applied in this research and one of them was female Bali cattle as a control. The remain of 23 male animals consisted of 10 Bali cattle from NTB/West Nusa Tenggara, 5 Bali cattle from Riau, 6 Bali cattle from Bali, 2 Bali cattle from Kalimantan and 1 Brahman as breed control collected from Sembawa, Palembang of south Sumatera. All DNA samples were collected from their fresh blood. Detail of material samples was summarized in Table 1.

Table 1. Material Samples Collected from Several Locations

No	Breed	No. Animal	Material	Location
1	Bali cattle	23	blood	Lombok, West Nusa Tenggara (10)
				Riau, Sumatera (5)
				Pulukan, Bali (6*)
				Pelaihari, South Kalimantan (2)
2	Brahman	1	blood	Sembawa, Palembang

*5 male and 1 female

DNA was extracted by using a method of a high concentrated salt (Montgomery and Sise, 1990; Mohammadi and Saberiva, 2009) while the small volume of blood samples was collected by using DNeasy Blood and Tissue Extraction kit (Qiagen).

UTY Primer. Primer targeting was re-designed from the reference sequence of AY936542.1 with full length target 484 bp of UTY 19 starting from nucleotide position 1 of the reference (AY936542.1).

Amplification and Sequencing. All DNA samples were amplified using designed primer UTY 19 F-5' TAA GTT GAA AGT TCT CTC ACT GTC 3'(24 bases) and R-5' CAA CGT TCA AAG TTG TTT ACA AAA 3' (24 bases) with targeting 484 bp of UTY-19. PCR reagent of AccuPower PCR Mix (Bioneer) was used with annealing temperature of 52^{o}C and run for 35 cycles. PCR products were visualized on 1% agarose gel using UV transilluminator exposure.

Amount of 6 selected PCR product samples of UTY gene fragments were sequenced through

a private company services. Those 6 samples represented 5 Bali cattle and 1 Brahman cattle, all samples were male cattle.

Sequence Analysis. The UTY gene sequence was aligned with the reference of UTY gene sequence *Bos gaurus* (AY936539.1), *Bison bison* (AY936540.1), *Bubalus bubalis* (AY936541.1), Bos taurus (AY936542.1), *Bos indicus* (Ginja *et al*. 2009) by ClustalW multiple alignment software. The UTY gene sequence was then analyzed with nBLAST from NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to see the similarity percentage (%) of UTY gene.

RESULTS AND DISCUSSION

UTY Gene and Purity Detection

All male samples showed a right size of the targeting UTY gene intron 19 of the Y-chromosome. Representative of targeting UTY fragment is presented in Figure 1.

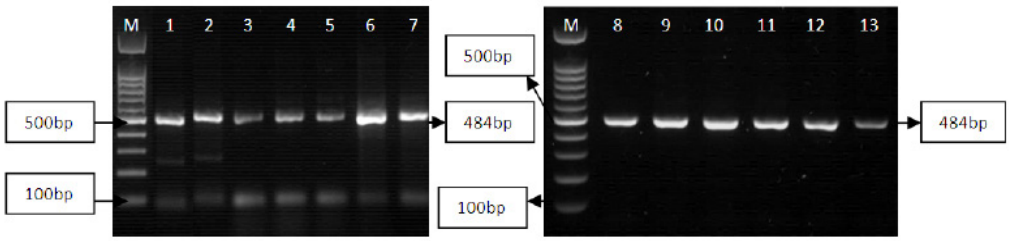

Figure 1. Representative PCR products of male animal samples bear UTY gene (484bp.) (M= 100bp. DNA Ladder; Lane 1 to 12= Bali cattle; Lane 13= Brahman)

The UTY gene exists in all male samples (Bali and Brahman male cattle) with fragment size of 484 bp. (Figure 1). This UTY gene was used for detecting the ancestor of modern cattle (Svensson and Götherström, 2008) and domestication (Götherström, *et al.*, 2005). This UTY gene is obtained only in male or exists only in Y-chromosome (Svensson, 2010; Liu and de Leon, 2007). Therefore, the UTY gene was not found in female Bali cattle (not presented in the Figure).

In the Figure 1 showed differences band pattern performance where lane 1, 2 and 6 got more than 1 band. This finding of ladder-like band (or smear) is similar to the previous study of Liu *et al*. (2003) where the unique band pattern was found in 17 bulls both individually and in other breeds. This ladder-like bands or multi-copy MS are unique for individuals or breeds. These multi copies features loci numbers. This multi-copy male specific sequence (MSY) can be used in Y-chromosome polymorphism analysis. In addition, individual bull got a unique haplotype Y (BTAY14) than can be used to identify individuals, breed or crossbreed (Liu and de Leon, 2007).

In the case of identifying purity of Bali cattle, we may need to explore with more specific genetic markers on the Y-chromosome. There are approximately 260 DNA markers, including ~50 MS,10 genes/ESTs, and ~200 BES, on the Bovine Y Chromosome (BTAY) map (Liu and de Leon, 2007). In addition, the future research might need to involve wild Banteng since it is believed that Bali cattle are originated from wild Banteng (Kusdiantoro *et al.,* 2009; Purwantara *et al*., 2012).

Nucleotide Variation

Nucleotide analysis of the Bali cattle and Brahman compared with UTY gene reference from genbank-NCBI by ClustalW Multiple Alignment is summarized in Table 2. The UTY gene references for the comparison were, *Bos gaurus, Bison bison, Bubalus bubalis and Bos taurus.*

Table 2. Summary of Sequence Alignment Analysis of UTY Gene in Male Bali Cattle Samples collected from Several Locations in Indonesia Compared to other Breeds from GenBank NCBI

No	Breed	Nucleotide Position															
		17	21	48	51	79	83	116	119	198	230	247-256	290	322	333	408	409
		Nucleotide															
1	01_Bima	C	A	C	C	A	T	T	C	T	C	-	C	A	G	A	A
2	04_Mataram	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.
3	05_Pulukan	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.
4	06_Riau	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.
5	07_Kalsel	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.
6	B. gaurus (AY.936539.1)	.	.	.	.	.	C	.	.	.	.	-	.	.	.	.	.
7	B.bison (AY.936540.1)	.	.	.	.	.	.	.	.	.	.	10bp*	.	.	.	.	G
8	B. bubalis (AY.936541.1)	A	T	T	A	G	-	C	T	C	A	10bp*	T	A	A	G	-
9	B. taurus (AY.936542.1)	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.
10	Brahman	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.

Remarks: * Insert Mutation= 10 bp. (AATACCAAAT); dots (…) = nucleotides not different among cattle breeds

Based on the Table 2, there were 16-nucleotide point differences between the five Bali cattle origin samples and other cattle breeds on the UTY 19. There were not found nucleotide base differences on the UTY 19 when Bali and Brahman cattle compared with reference UTY 19 of *Bos taurus* (AY 936542.1). It might be the origin of Bali cattle and Brahman cattle are from tropical area while *Bos taurus* is typically origin cattle breed from Europe with cold weather. The Y-Chromosome is paternally inherited it has been used to study the evolution and migration patterns of modern humans by using Y-Chromosome haplotypes (Quintana-murci *et al*., 2001).

Similarity analysis of the sequenced cattle samples to the reference UTY gene is presented in Table 3. Those references used for comparison were *Bos taurus, Bison bison, Bubalus bubalis* and *B. indicus.*

Table 3. Simmilarity UTY Sequence of Bali Cattle Samples from different locations Compared to other Breeds

Breed	Sample Location	Similarity	Gaps	Bases change	Length of sequence (bp)	Acc. number	References
B. taurus clone SUTY		100%	-	None	415	AY936542.1	Gotherstrom *et al.,* 2005
Bison bison		97%	10 bp	at 247	494	AY936540.1	Gotherstrom *et al.,* 2005
Bubalus bubalis		95%	10 bp	at 247	494	AY936541.1	Gotherstrom *et al.,* 2005

B.indicus (Brahman)		100%	-	Short base	280	Brahman	Ginja *et al.,* 2009
B.indicus (Brahman)	Sembawa, Palembang	100%	-	None	420	-	This research

In the Table 3 showed 100% similarity when Bali cattle (from 5 locations) compared to *Bos taurus* (AY936542.1; Gotherstorm *et al*. 2005), 100% when compared to *Bos indicus* (Ginja et al. 2009) and 100% compared to Brahman (*Bos indicus*) of this research. However, the similarity to UTY gene was 97% when the Bali samples compared to Bison bison (AY936540.1; Gotherstorm *et al.* 2005) and 95% when compared to *Bubalus bubalis* (AY936541.1; Gotherstorm *et al.* 2005). There was insertion of 10 nucleotides at the position of 247 in *Bison bison* and *Bubalus bubalis*. Therefore the nucleotide numbers of those breeds are longer 10 nucleotides compared to the UTY of Bali cattle samples, *Bos taurus*, *Bos gaurus* and *Bos indicus*. All references belong to order Artiodactyla, suborder Ruminantia, family Bovidae (Prusak *et al.*, 2004), however still had small difference of UTY gene similarity in *Bison bison* (97%) and *Bubalus bubalis* (95%).

CONCLUSIONS

All male DNA samples bear UTY gene intron 19 of Y-chromosome. There is not found nucleotide variation in the UTY gene fragment of Bali cattle and Brahman males compared to the reference of *Bos taurus* (AY936542.1). There is found 16 points of nucleotide variation in the UTY gene fragment of Bali cattle compared with reference of *Bos gaurus* (AY936539.1), *Bison bison* (AY936540.1) and *Bubalus bubalis* (AY936541.1). The existing UTY gene in the intron 19 of Y-chromosome could not be used to determine the purity of Bali cattle breed. In coming research, the study will focus on the specific marker in the Y-chromosome for identifying individual breed and the crossbreed of possibility introgression of other breed in Bali cattle. So, it might be can explain clearly the breed purity.

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