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Genetic Diversity of Indonesian Native Cattle Based on Y-Chromosome Microsatellite and its Like-Ladder DNA Conformation

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Abstract: We used microsatellite DNA marker of the Y-chromosome to know the polymorphism of this marker on Indonesian native cattle populations because the Y-chromosome marker was required as an analog line male as well as a mitochondrial DNA (mtDNA) line in female. The cattle used in this study were 25 heads unrelated male animal for each breed and total samples were 175 heads for seven breeds. The highest number of alleles was two alleles were found in all of Y-microsatellite locus whereas the average number of alleles in all populations (the seven breeds of cattle) was 1.1 and the overall number of alleles was quite low (1-2 alleles). The values of heterozygosity for seven breeds were 0 up to 53%. The highest heterozygosity (h) value of microsatellitte loci was found on the INRA 062 (53%) locus in the Pesisir cattle (West Sumatra local cattle) population. While, the PIC values for all of microsatellites between 0.10-0.29 and the highest value was found on Madura cattle.

Key words: Y-chromosome microsatellite, Bali cattle, Banteng, DNA, allele

INTRODUCTION

The genetic studies of Indonesian native cattle have interested since Indonesia has a great genetic variation of native cattle. For instance, Bali cattle was the most popular native cattle comparing other breeds because these cattle has been known as the one of Banteng (Bibos banteng) descendant. In the other hand, we also could find others native cattle of Indonesia that suspected to have genetic status descendent from Banteng (i.e., Madura cattle) where might be introducing genetic by modern breeds like *Bos indicus* or *Bos taurus*. So, this was important more study for Indonesian native cattle regarding to genetic improvement or genetic preserved of Indonesian native cattle. Because, it could prevent from decreasing or extinction of the specific characters of Indonesian native cattle.

The various techniques and studies for genetic characterization of Indonesian native cattle were carried out based on blood type and protein (Namikawa *et al*., 1980), blood proteins and enzymes and composed of the amino acid β-chain from haemoglobin X. These results indicated that Bali cattle have a specific character, like HbX allele of blood where have not found on others, especially on Zebu and Taurine breeds. Information about specific alleles or breed specific allele from molecular data on Indonesian native cattle is still limited. The specific allele or breed specific allele from short tandem repeat at locus INRA 023 of Bali cattle was reported. Also, Bali cattle has a specific allele at locus HEL9 and INRA 035 microsatellite comparing to *Bos taurus* (Simmental, Limousin and Brangus) breed. So, this study only limited finding about specific allele of Indonesian native cattle. Based on 16 microsatellite markers (Winaya, 2000), DNA microsatellite was able to identify the genetic relationship among native cattle like Bali and Madura cattle and others from Indicus (Ongole) and Taurus (Brangus).

In this study, we used microsatellite DNA marker of the Y-chromosome to know the polymorphism of this marker on Indonesian native cattle populations. Therefore, the Y-chromosome microsatellites only specific for male, we hoped this marker could be illustrated the male genetic characters. Because, it has been known that male could play an important role on the genetic transmission of characters that owned by the ancestor to

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his descendants. Beside that, Bali cattle as the one of Banteng descendant that represented by the Indonesia indigenous breed need to prevent from extinction. In the other hand, the analysis of the genome and population genetics were led to haplotype Y-chromosome analysis where was an important tool in studying population by naturally (Hurles and Jobling, 2001).

Y-chromosome was only a part of the mammalian genome that was exclusively derived from the paternal line or patrilineality. Therefore, the Y chromosome was the unique marker for studying animal contribution to the evolutionary history of the male of a species. For instance, genetic marker that associated with the Y-chromosome has contributed as consideration in the history of human phylogeography (Hammer *et al*., 1997). However, the application data on the Y-chromosome genetic populations of non-primates, such as horse, cattle and sheep were still very rare due to the rarity of the marker on the Y-chromosome and its sequence information (Petit *et al*., 2002) as well as low level variations (Hellborg and Ellegren, 2004; Meadows *et al*., 2004; Queney *et al*., 2001). So, it could be stated that various combinations on the occurrence of mutations throughout the male lineage was a conserve and a single haplotype-linkage that rarely deviate or unbiased. Analysis of genetic variations in this area was assumed more accurate to expect the male cattle characters.

The Y-chromosome marker was required as an analog line male as well as a mitochondrial DNA (mtDNA) line in female. Levels of polymorphism on the area of non-recombinant Y-chromosome, starting from the lowest or rare that the occurrence of bi-allele mutation at the point of Single Nucleotide Polymorphisms (SNPs) until the most commonly found on the minisatellite or microsatellite locus marker (short tandem repeat STR). While, the polymorphism of SNPs on Y-chromosome was often found in specific populations (Hammer *et al*., 1997).

MATERIALS AND METHODS

Selection of animals: The cattle used in this study were 25 heads unrelated male animal for each breed and total samples were 150 heads for seven breeds.The age animals from 1.5-2.0 year old and the sex were male. Samples were obtained from Artificial Insemination (AI) regional office in Baturiti District and center for development of promising Bali cattle, Jembrana District, Bali Province and AI regional office at Gunungsari, Lingsar District, West Nusa Tenggara Province of Bali cattle. While, other samples were came from aceh province of aceh cattle, pesisir cattle from Pesisir District of West Sumatra province, Ongole and Holstein crossbred from East Java Province.

Blood cell collection: Blood samples of cattle were collected 10 mL for each male. Blood cells were obtained using venoject from jugularis vein. This blood cell then mixed with EDTA 10% for preservation and anti coagulant until, used for source of the DNA genome through isolation processes.

DNA extraction: Genomic DNA was isolated from blood cells using standadrized protocol of phenol-chloroform extraction technique (Sambrook *et al*., 1989). Quality and quantity of extracted DNA were checked with horizontal submarine mini electrophoresis in 0.8% agarose, using 0.5X, TBE as running buffer and those were measured the Optical Density (OD) at 260 and 280 nm wave lenght in UV-VIS spectrophotometer and steril water was used as a blank sample.

Primer and optimization of Polymerase Chain Reaction (PCR): The primer pairs were used in this research Y-chromosome locus microsatellite of *Bos taurus* sequence. PCR amplifications were carried out in the thermal cycler machine (PCR machine) programmed according to each primer pair. The composition of the master-mix as followed: 60 ng of template DNA; 0.8 units of Taq polymerase; 0.2 mM of dNTPs; 0.5 μmol primers (forward and reverse) and 1 X PCR buffer with 1.5 mmol $MgCl_2$ (Table 1).

Polyacrylamide gel electrophoresis: Five microlitters (μL) of PCR product mix with 8 μL of loading dye and then,

Table 1: Microsatellite markers, their sequence, size range and references

Locus	Primer sequence (5'-3')	Size range	References
DYS3 (INRA008)	GAG CCT GTG TGT GTA TAC AC (GGC ACT TTC CTC TCC TGT CGC G)	140	Vaiman *et al*. (1994)
DYS4 (INRA057)	CCT AGC GAC TGT CCA AGC G (CAC GGG CTG AGA ATT CAA AAC)	125	Vaiman *et al*. (1994)
DYS5 (INRA062)	TGT GCA GCA CCT TGT CTC C (ACA TGC ATG TGC TTG TGT CG)	150	Vaiman *et al*. (1994)
DYS6 (INRA124)	GAT CTT TGC AAC TGG TTT G (CAG GAC ACA GGT CTG ACA TG)	130-132	Vaiman *et al*. (1994)
DYS7 (INRA126)	TCT AGA GGA TCA AGG ATT TGT G (AAT CCA TGG AAA GAT GCA CTG)	182-186	Vaiman *et al*. (1994)
DYS199 (M3)	AAT AGG TTG ACC TGA CAA TGG (TCA TTT TAG GTA CCA GCT CTT)	113	Underhill *et al*. (1996)
INRA189	TAC ACG CAT GTC CTT GTT TCG G (CTC TGC ATC TGT CCT GGA CTG G)	68-124	Kappes *et al*. (1997)

electrophoresed on a polyacrylamide gel in vertical electrophoretic. The different concentrations of the non-denaturing gels (10, 12 and 14%) were used for amplicon separation depending on the size of the amplicon. Gel then, runs at a constant voltage (200 V) for 8-12 h (depending on amplicon size) at 4°C. The gel then fixed in 10% of glacial acetic acid for 30 min and staining with freshly 1% silver nitrate solution for 40 min or until, DNA bands appears with dark brown colour. Gel then immediatelly, transferred to the freshly prepared developing solution (3% sodium carbonate, 10 mL formaldehyde, 0.001% sodium isothiocyanate) and followed by a brief washing in distilled water. The various band pattern of the amplified PCR products will be marked and score by manually (Leung *et al*., 1993).

Statistical analysis: The band that appears in polyacrylamide gel which stained silver staining at each locus was assumed as the DNA microsatellite allele. The diversity of microsatellite alleles were determined from the difference of allele migration in the gel for each individual sample. Then, the frequency of each allele of each microsatellite loci was calculated according to the formula:

$$X_i = \frac{2n_{ij} + \sum n_{ij}}{2n}, \ldots, j \neq 1$$

Where:

x_i = Allele frequency i
n_{ij} = Individual number for genotype ij
n = Allele number

The genetic diversity measured by the mean value of heterozygosity (h) on all of locus, both polymorphic and monomorphic locus. The locus polymorphic when allele frequency that was obtained equal to or <0.99 (Nei, 1987). Equation for obtaining the value of the heterozygosity (h) for each locus were:

$$h = 2n\frac{1 - \sum Xi^2}{2n - 1}$$

Where:

h = Heterozygousity of locus
x_i = Allele frequency of locus ith
n = Number of individual sample

The phylogenetic trees then constructed by following Unweighted Pair Group Method for Arithmetic mean (UPGMA) for 1000 bootstrap values (Sokal and Sneath, 1963).

RESULTS AND DISCUSSION

The heterozygosity and Polymorphic Information Content (PIC) of Y-chromosome microsatellite: The genetic diversity of Indonesia native cattle breeds was determined by analyzing the type and number of alleles, heterozygosity and Polymorphic Information Content (PIC) in Y-chromosome microsatellites. The highest number of alleles was two alleles that were found in all of microsatellite locus whereas the average number of alleles in all populations (the seven breeds of cattle) was 1.1 and the overall number of alleles was quite low (1-2 alleles).

The values of heterozygosity for seven breeds were 0 up to 53% in Table 2. The highest heterozygosity (h) value of microsatellitte loci was found on the INRA 062 (53%) locus in the Pesisir cattle (West Sumatra local cattle) population Table 3. While, the PIC values for all of

Table 2: Frequency of microsatellites alelles on Y-chromosome of cattle

	Aceh			Pesisir			Madura			Bali-bali			Bali-lombok			PO			PFH		
LOCUS	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
INRA008	18	2	A = 3(0.17) B = 15 (0.83)	15	1	A = 3(0.20) B =12 (0.80)	18	2	A = 5(0.28) B = 13(0.72)	18	2	A = 6(0.33) B = 12(0.67)	18	2	A = 2(0.11) B = 16 (0.89)	18	2	A = 13(0.72) B = 5(0.28)	18	1	A = 18 (1.0)
INRA057	18	15	A = 18 (1.0)	18	2	A = 2 (0.13) B = 13(0.87)	18	2	A = 5(0.28) B = 13(0.72)	18	2	A = 2(0.11) B = 16(0.89)	18	2	A = 4(0.22) B = 14(0.78)	18	2	A = 15(0.83) B = 3(0.17)	18	1	A = 18 (1.0)
INRA062	18	2	A = 6(0.33) B = 12(0.67)	15	2	A = 7(0.47) B = 8(0.53)	18	2	A = 4(0.22) B = 14(0.78)	18	2	A = 3(0.17) B = 15(0.83)	18	2	A = 2(0.11) B = 16(0.89)	18	2	A = 10(0.56) B = 8(0.44)	18	1	A = 18(1.0)
INRA124	18	2	A = 5(0.28) B = 13(0.72)	15	2	A = 3(0.20) B = 12(0.80)	18	2	A = 5(0.28) B = 13(0.72)	18	2	A = 8(0.44) B = 10(0.56)	18	2	A = 6(0.33) B = 12(0.67)	18	2	A = 11(0.61) B = 7(0.39)	18	2	A = 15(0.83) B = 3(0.17)
INRA126	18	1	A = 18(1)	15	2	A = 1(0.07) B = 14(0.93)	18	2	A = 3(0.17) B = 15(0.83)	18	2	A = 2(0.11) B = 16(0.89)	18	2	A = 4(0.22) B = 14(0.78)	18	2	A = 14(0.78) B = 4(0.22)	18	2	A = 13(0.72) B = 5(0.28)
DYS199	18	2	A = 3(0.17) B = 15(0.83)	15	1	A = 15(1.0)	18	2	A = 4(0.22) B = 14(0.78)	18	2	A = 3(0.17) B = 15(0.83)	18	2	A = 4(0.22) B = 14(0.78)	18	2	A = 12(0.67) B = 6(0.33)	18	2	A = 18(1.0)
INRA189	18	2	A = 4(0.22) B = 14(0.78)	15	1	A = 15(1.0)	18	2	A = 4(0.22) B = 14(0.78)	18	2	A = 2(0.11) B = 16(0.89)	18	2	A = 2(0.11) B = 16(0.89)	18	1	A = 18(1.0)	18	2	A = 1(0.06) B = 17(0.94)

Table 3: Heterozygousity (h) and Polimorphic Information Content (PIC) of Y-chromosome microsatellite DNA on cattle population

	Aceh		Pesisir		Madura		Bali		Lombok		Ongole		FH	
LOCUS	h	PIC	h	PIC	h	PIC	h	PIC	h	PIC	h	PIC	h	PIC
INRA008	0.30	0.24	0.34	0.27	0.43	0.32	0.47	0.34	0.21	0.18	0.43	0.32	0.00	0.00
INRA057	0.00	0.00	0.24	0.20	0.43	0.32	0.21	0.18	0.36	0.28	0.30	0.24	0.00	0.00
INRA062	0.47	0.34	0.53	0.37	0.36	0.28	0.30	0.24	0.21	0.18	0.52	0.37	0.00	0.00
INRA124	0.43	0.32	0.34	0.27	0.43	0.32	0.52	0.37	0.47	0.34	0.50	0.36	0.30	0.24
INRA126	0.00	0.00	0.14	0.12	0.30	0.24	0.21	0.18	0.36	0.28	0.36	0.28	0.43	0.32
DYS199	0.30	0.24	0.00	0.00	0.36	0.28	0.30	0.24	0.36	0.28	0.47	0.34	0.00	0.00
INRA189	0.36	0.28	0.00	0.00	0.36	0.28	0.21	0.18	0.21	0.18	0.00	0.00	0.12	0.11
Mean	0.27	0.20	0.23	0.17	0.38	0.29	0.32	0.25	0.31	0.25	0.37	0.27	0.12	0.10
SD	0.20	0.14	0.19	0.14	0.05	0.03	0.13	0.08	0.10	0.06	0.18	0.13	0.18	0.13

micro satellites between 0.10-0.29 and the highest value was found in Madura cattle but, this value was low polymorphic according to Botstein *et al*. (1980) (Table 3).

The phylogenetic relationship based on Y-chromosome microsatellite: The genetic relationship (phylogenetic) analysis based on Y-chromosome microsatellite markershowed that Bali cattle (from Bali Island) and Madura cattle had the closest genetic distance (71%) compared to Bali cattle from Lombok Island population (64%). But, these breeds were still in one cluster. While, Pesisir cattle from West Sumatra Province was closest cluster tothese three breeds. On the other side, Ongole and FH offspring were grouped in one cluster with a branch node on Aceh cattle from Aceh Province.

Y-chromosome microsatellite polymorphism: The polymorphism of microsatellite markers that indicated by heterozygosity (h) value were low (from 0 up to 53%). This result was different with the previous study by Li *et al*. (2007) that studied on the Ethiopia cattle used five microsatellites locus of Y-chromosome (INRA124, INRA126, INRA189, BM861 and BYM-1) where the values of heterozygosity were 0.541 (54.1) up to 0.795 (79.5%). These results indicated that the genetic diversity of Ethiopia cattle was still quite high compared to Indonesia native cattle. These differences condition might be approached by several potential factors. Because of this study used microsatellites marker of the Y-chromosome, the lowest average number of alleles on the Y-chromosome could be affected by selection factor, mating system, or migration patterns as well as other mechanisms that result in a low number of males for effective population size (Meadows *et al*., 2006).

Other studied by Ginja *et al*. (2009) which analyzed genetic variations on Y-chromosome used Portuguese cattle (*Bos taurus*) and Brahman cattle (*Bos indicus*) based SNPs and STRs (short tandem repeat) on Y-chromosome. The heterozygosity value (h) average on Portuguese cattle between 0.09 (9) up to 0.30 (30%) and Brahman cattle was 0 (0%). The Y-chromosome was non-recombinant region which was effective to receptive the influence of selective pressure due to the action of a specific sequence on the chromosome with the exception of pseudo-autosomal regions. This condition was very contrary to the situation in the autosome and X-chromosome where any recombination events could result a new DNA sequences from several locus compositions (Nowak, 1991).

According to, FAO (2004) guidelines to assess the genetic variation between breeds should have at least four alleles different per locus. So, in this study all of the loci were still not appropriate for these criteria. Therefore, in this case, we suggested adding more number of samples and geographical location wider for analysis of genetic variation among breeds. This was assumed that more samples in this research expected to find other alleles that could be justified as polymorphic alleles as well as the provisions of FAO (2004). In fact, we have not found more allele yet. This condition could be presumed that might influence by another factor like inbreeding. Because, in general the management of animal mating, especially on Artificial Insemination (AI) was lead used limited bull. On the other hand from heterozygosity value that exceeded 50%, mean that the genetic diversity in the population was quite high. So, if the number of individual population could be added, it would increase the level of diversity in the population that was observed. It's expected to find the specific candidate markers for Indonesia native cattle based on Y-chromosome microsatellite marker. This was important because the status of the males as a transmitter of specific genetic character was still needed, especially related to AI management in Indonesia.

However, in this study, also found the interesting phenomenon that some individuals of Bali cattle have double alleles or multiple alleles or multi-allelic in similar locus, like INRA 057 and INRA 189 locus (Fig. 1-3). Theoretically, this phenomenon should not be occurring because Y-chromosomal microsatellite allele is a haplotype model. But, according to Li *et al*. (2007) that the events of the multi allelic and a form of like-ladder DNA

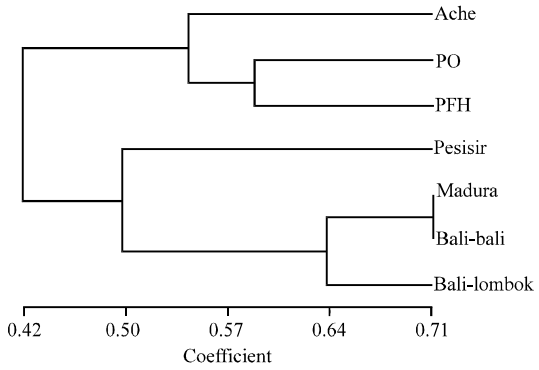

Fig. 1: The phylogenetic diagram of Indonesia native cattles based on Y-chromosome microsatellite DNA marker. (PO = Ongole offspring, PFH = Frisian Holstein offspring)

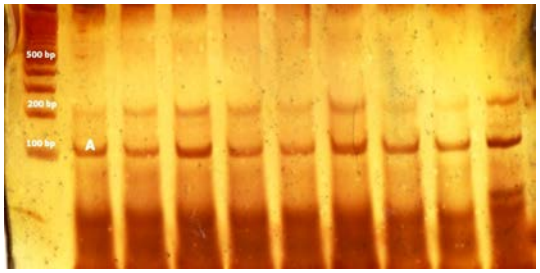

Fig. 2: PCR products stained by silver from INRA 189 locus of Y-chromosome microsatellite on Bali cattle. (M = 100 bp ladder; 1-9 = individu samples; A = alelle microsatellite). Here, the Y-chromosome microsatellite band allele are double (multi-alellic or multi-copy) or called hemyzygous

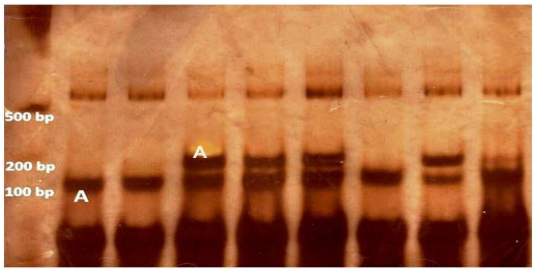

Fig. 3: PCR product stained by silver from INRA 057 locus of Y-chromosome microsatellite on FH cattle (M = 100 bp ladder; 1-8 = individu samples; A = Alelle microsatelite). Here, any DNA multi-allelic (multi-copy) events

(ladder-like bands) was one of the unique events of Y-chromosome microsatellite that called the multi-copy or hemyzygous event of microsatellite loci. Like Edwards *et al*. (2000) studied also found a few multi-alleles at locus of Y-chromosome microsatellite, i.e., on the INRA 126 of *Bos taurus* and *Bos indicus* cattle.

According to Li *et al*. (2007), the cause of double or multiple alleles (multiple-copy allele) were microsatellite loci lies in the Pseudo Autosomal Regions (PAR) or not lies in the Y-Specific Male (MSY) region. It has been known that, the PAR area was the end-region of the Y-chromosome that its inheritance model similar to the autosome region. Thus, the male has two copies of the gene or DNA sequence on its Y-chromosomal region; one set in the PAR and the other set of its pair in the X-chromosome. So, males can be inherited his allele on the X-chromosome from his father and female can inherit her alleles on the Y-chromosome from her father.

Perez-Lezaun *et al*. (1997) and Li *et al*. (2007) also proved that Y-chromosome microsatellite on INRA 124 and INRA 126 locus could be amplified on females. This means that the alleles of INRA 124 and INRA 126 locus probably could result multi-allelic events because these alleles were also found in X-chromosome of females. So, Perez-Lezaun *et al*. (1997) not recommended these two microsatellites when used in the study of genetic characterization of the incident on the males introduction. Then, Edwards *et al*. (2007) also reported that INRA 126 microsatellite could be amplified both in male and female on the Yak (Bos grunniens). This was also indicated that INRA 126 locus had a homologous sequence on the X-chromosome. So, the allele phenomenon with double or multi-copy alleles that found in this study was the one of the phenomena that Y-chromosome microsatellite alleles also had this uniqueness. However, in the determination of the genetic variation, the double or multiple-allele copy was still determined as one type of allele because the other allele is only duplicated or the copy of the real allele. This event could be explained by the consequences of the segregation and recombination of chromosome events approaching, especially on the part of the PAR of Y-chromosome where such unique events occurred.

The genetic relationship of Indonesian cattle based on Y-chromosome microsatellite: Based on the genetic relationship or phylogenetic analysis, it appeared that Bali cattle from Bali Island and Madura cattle have the closest genetic distance (71%) compared to Bali cattle (from Lombok Island). However, these three breeds of Indonesia local cattle were still, in one cluster. It means that, these breeds (Bali-Bali, Madura and Bali-Lombok) have a genetic closeness based on seven locus alleles of Y-chromosome microsatellite that were tested. Although, in different cluster, Pesisir cattle from West Sumatra Province have closest genetic distance with

Indonesia three breeds cluster. So, it could be assumed that Pesisir cattle might also, genetic closer relationship to Bali and Madura cattle than Aceh cattle. Although, Aceh cattle stated also as Indonesian native cattle but in this study, Aceh cattle was in a similar group with Ongole and FH offspring. This could be assumed that Aceh cattle might also have a specific locus of Y-chromosome microsatellite from *Bos indicus* (i.e., INRA 124, 126 and 189 INRA INRA). Generally, Aceh cattle phenotypes are close to Ongole breed or *Bos indicus* for instance in colored skin and humped. Thus, it was assumed that more proportion of genetics of Aceh cattle came from *Bos indicus* breed.

On the other side, Ongole and FH off spring were grouped in one cluster with a branch node on Aceh cattle. It was also assumed that Aceh cattle might have any types or number proportion of alleles coming from both *Bos taurus* and *Bos indicus* and might also mix between those breeds. FH breed was a *Bos taurus* and Ongole was a *Bos indicus*. But, based on this study the branch of genetic distance between Aceh cattle and those two breeds were far enough (<50%). So, it was presumably that the genetic variation between Aceh cattle and Ongole and FH cattle was far enough. So by using specific marker of Y-chromosome, DNA microsatellite, generally able to give a description about the genetic relationship between local cattle that existing in Indonesia. Because, there were no markers that indicated the PIC value >50% so it still, needs more number of similar markers for further study to describe more clearly about the genetic relationship of Indonesia native cattle.

The value of Polymorphic Information Content (PIC) of the Y-chromosome microsatellite DNA marker showed that values start from 0-0.37 with a PIC mean value from 0.10-0.27 and from the seven loci, we did not find locus that has PIC value >0.50. So, according to Botstein *et al.* (1980), seven loci that were used in this research were categorized as a less informative locus for analysis of population genetics. Meadows *et al.* (2006) also found that a low value on the variations of the nucleotide sequence in a specific region of the Y-chromosome in some species of animals included cattle. So, based on this study still needed further research to find more information about polymorphic locus of the Y-chromosome microsatellite marker on Indonesia native cattle. But, from this previous studied, we expected that this research could be added as useful information for exploring the genetic variation of Indonesia native cattle.

Studied by Cai *et al.* (2006) in China native cattle used two microsatellites locus, namely UMN2404 and UMN0103, showed that each marker only has two alleles. It presumably came from *Bos indicus* and *Bos taurus* breeds. It means that China cattle might be a mixture from both breeds. Also, there were many studies suggested that China cattle were descended from Bos primigenius species (Chen *et al.*, 1995). Because at the last time, a Mongolian tribe probably introduced to China land also brought the cattle. They were domestication of wild cattle from Primigenius breed and then crossed with *Bos taurus* breeds. This factor might be assumed causes of genetic mixed on China native cattle.

The similar with Indonesian native cattle which little bit different situation from China cattle. The introduced of *Bos taurus* and *Bos indicus* genes were occurring because of political factor when the colonial government rule Indonesia at last decades ago. For example, the case of Ongole breed that imported from India and then was raised on Sumba Island of the West Nusa Tenggara Province. This island was used as a place of purification for this breed because at that time was suitable for development of this breed. Finally, this breed is now be a part of Indonesia native cattle. So, in general could be stated that the genetic composition of Indonesia native cattle presumably a mixture between Banteng (Bibos banteng) and *Bos taurus* and *Bos indicus*.

Also, based on early studied that using blood type Namikawa *et al.* (1980), protein (Noor *et al.*, 2000), satellite and microsatellite DNA autosome (Winaya, 2000; Verkaar *et al.*, 2002, 2003; Nijman *et al.*, 2003; Ugla, 2008) and mitochondrial DNA (Verkaar *et al.*, 2002, 2003; Ugla, 2008; Mohamad *et al.*, 2012), all had been proven that the one important of genetic components of Indonesian cattle came from Banteng. Thus, it had putative that Banteng and Indonesiannative cattle had the closest relationship based on the genetic distance from phylogenetic analysis. Based on this study if will determine the specification of Indonesia local cattle, we suggested could use this reference with to the reason that specification characters of Indonesia native cattle, included Bali, Madura, Aceh and Pesisir cattle should have greater in genetic proportion from Banteng. Besides, it had been known that based on the historical findings, both from animal fossils remains and historical records, Banteng had become the one of cattle breed ancestors in the world.

CONCLUSION

The analysis of genetic variation in Indonesiannative cattle using molecular markerof Y-chromosome microsatellite DNA could explain not only the low variation on this marker that were used but also find the phenomenon that was named multiple alleles (multi-allelic) at certain loci on the Y-chromosome microsatellite. But,

this condition would not become problematic as long as not changing in gene expression because this marker is not a gene marker but microsatellite marker (or accessories DNA) of course will not impact on protein expression.

The existence of Indonesiannative cattle was influenced by introduction of gene flow from *Bos taurus* and *Bos indicus*, included Bali, Madura, Aceh and Pesisir cattle. However, Bali cattle had greater character that introduced by Banteng (Bibos banteng) generally. As suggested, Indonesiannative cattle might be should content Banteng genetic proportion. However from this study generally, the polymorphism of Y-chromosome microsatellite DNA was low. So, it still needs further research by considering more sample number and wider geographical location. Bali and Madura cattle have been known to have the largest proportion of genes from Banteng, thus both cattle can still be existence as a native cattle of Indonesia. While, Aceh and Pesisir cattle are have had a great opportunity for enhanced their existence as a specific cattle based on its regions (Aceh and West Sumatra Province) eventhough, the genetic proportion more *Bos taurus* and *Bos indicus* characters.

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