

## Contributions of *Bos Indicus* Breed to Genetic Diversity of Sumatra Native Cattle Based on Y-Chromosome Microsatellite Marker

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### ABSTRACT

The genetic composition of cattle in the world generally tends to two dominated breeds, *Bos taurus* (European cattle) and *Bos indicus* (Asian cattle). From morphological phenotype could be differed by hump for Asian cattle and humpless for European cattle. Molecular study indicated that any alleles or haplotypes which tend to those dichotomy. Similar condition in Indonesian cattle, including Aceh and Pesisir cattle, previous study showed that any indication introduction of Taurine and Indicine breeds in those cattle, while in the other hand Indonesian native cattle is descendent from the one of common ancestor cattle in the world, Banteng. For advancing study in genetic introduction of other breeds in Aceh and Pesisir cattle, we assayed those Sumatra's breeds by using molecular marker of Y-chromosome microsatellite. The using of Y-chromosome marker by assumption could be a model for detection of male introduction in breed. From this research showed that all of locus have low allele number, both in Aceh and Pesisir cattle. Also from *Polymorphic Information Content (PIC)* value, this marker has lower value (less 0.5) than FAO recommended. But, in these result indicated that *B. indicus* is the one of the genetic composition of Aceh and Pesisir cattle. Because locus INRA 124 was could amplification in those breeds and these locus also the one of *B. indicus* specific allele.

*Key words: Bos taurus, Bos indicus, Bos javanicus, Y-chromosome microsatellite, Aceh cattle, pesisir cattle*

### INTRODUCTION

Asia continent has more or less three hundred millions cows and two hundred millions of them reside in India sub-continent. Cattle breeds in Asia and Africa are generally categorized into hump and humpless. About 170 breeds are already known, including Bali cattle (domesticated from Banteng) in Southeast Asia, including Indonesia and Philippine. So, Asian farmers have been famous in their role in cattle domestication process and agriculture ecology in this continent (Schearf, 2003).

Bali cattle and other breeds have genetic relationship with Banteng (just like Madura cattle) and also have genetic admixture with *B. taurus* and *B. indicus*. Because of economic and political factors in the past (during colonialism era or after the independence), they are brought to Indonesia and finally could adapt to the environment and become part of local cattle. Aceh cattle are believed to be the local breed, but the previous studies by Muhamad *et al.* (2007) and Uгла (2008) shows that the genetic

compositions of those cattle came from *B. indicus*. So, the genetic study of native cattle in Indonesia is interesting because the genetic variations are great. This is important because it is related to the efforts characteristics improvement and keep genetic characters conserved. Finally, the quality of those native cattle does not decrease or even extinct.

The recent development on genome and genetic analysis on human population shows a tendency to haplotype of Y-chromosome which is an important tool in studying population naturally (Hurles & Jobling, 2001). Without neglecting pseudo-autosom, Y-chromosome action in general is a non-recombinant unit, which is male specific and effective haploid. This is to make sure that the combinations mutation along male offspring is concentrated as a single un-biased haplotype. Y-chromosome characters are needed in its context as male lineage just like mitochondrial DNA in female lineage. The level of polymorphism characters in non-recombinant area of Y-chromosome area is started from the lowest, that is biallele event in point mutation of

single nucleotide polymorphisms (SNPs) to the most common event in locus characters minisatellite or microsatellite (Short Tandem Repeat [STR]). Eventhough, the polymorphism of SNPs in Y-chromosome is often founded in specific population (Hammer *et al.* 1997). However, data of Y-chromosome in non-primate (like cattle, goat and lamb) genetic population is rare because of its rare marker in Y-chromosome in sequence information (Petit *et al.* 2002) and its low variation level (Hellborg & Ellegren 2004; Meadows *et al.* 2004; Queney *et al.* 2001).

Domestic cattle show indicated that individual variation into population based on microsatellite molecular marker is specific character to their sex chromosome (Y-chromosome), the existence of breed hybridization and their migration (McHugh *et al.* 1997; Bradley *et al.* 1998). Therefore, the existence of polymorphism in microsatellite specific to Y-chromosome can be used as a starting point in providing information about paternal genetic variation study on cattle or related species. The indication that growth hormone pseudogen (GH) is found in male domestic cattle shows that sex chromosome can influence individual differentiation process. It is important to know the role of sex chromosome in male cattle, for examples, in their reproduction ability.

Hanotte *et al.* (2000) study using microsatellite characters in Y-chromosome of INRA23 locus showed that there is any introduction of Zebu male in local cattle, Mozambique and Zimbabwe. It is suspected that their alleles came from Mozambique bay. In the past, Mozambique bay is an international trading area including cattle trading, so that was able to make genetic introduction of cattle from other breeds.

Edward *et al.* (2000) study using four characters microsatellite DNA marker (INRA124, INRA126, INRA 189 and BM 861) showed an evidence that any male genetic flow from *B. Taurus* or Zebu on hybrid population of bovidae species, including cattle. A study on African cattle using microsatellite DNA on Y-chromosome showed that there is a high different in cattle in central Africa and Southwest Africa.

The specific aim of this study is to know the pattern of Y-chromosome microsatellite DNA allele polymorphism in Aceh and Pesisir cattle, and to get Y-chromosome microsatellite DNA specific alleles of Aceh and Pesisir cattle.

## MATERIALS AND METHODS

### Collection of DNA Samples

DNA was extracted from total blood-cells which were collected from cattle as many as 10 ml per sample and preserved using EDTA 10%. The two areas where the samples came from were Aceh cattle from Breeding Center of Promising Aceh Cattle (*Balai Pembibitan Ternak Unggul (BPTU) Sapi Aceh*), Indrapuri Sub District, Aceh Besar Regency, Nangroe Aceh Darussalam Province and Pesisir cattle from Painan Sub District, Pesisir Selatan Regency, West Sumatera Province.

### DNA Amplification and Allele Microsatellite Detection

DNA extraction using phenol-chloroform standard method (Sambrook *et al.* 1989) was then preserved in TE buffer. Locus-microsatellite amplification used seven primers which is flanks Y-chromosome microsatellite locus (INRA008, INRA057, INRA062, INRA 124, INRA 126, DYS 199, and INRA 189). Microsatellite allele PCR product were separated using PAGE 10% and observation was done manually (Leung *et al.* 1993) after silver staining (Tegelstrom 1986).

### Y-Chromosome Microsatellite Allele Analysis

Microsatellite alleles were obtained from observation result and were analyzed statistically to get frequency and distribution value of allele, heterozygosity (*h*) allele and *Polymorphic Information Content* (PIC) in sample population.

Total number and frequency of microsatellite alleles were calculated to get the value of heterozygosity and alleles frequency per locus using formula:

$$f(A) = \frac{A}{2n}$$

where:

f (A) = Frequency of microsatellite allele

A = Total number alleles of i<sup>th</sup> locus

N = Total number of individual observed

Locus is considered polymorphic if the value of f(A) is the same or less than 0,9. Genetics variation is determined from the average value of heterozygosity (*h*) for all locus.

Heterozygosity value per locus is calculate according to formula bellow (Nei 1987):

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

where:

$h$  = locus heterozygosity

$Xi$  = alleles frequency of  $i^{th}$  locus

$N$  = total number of individu observed

Allele frequency is also used to get Polymorphic Information Content (PIC) index, i.e. value to calculate marker informative level, calculated according to Botstein *et al.* (1980) formula:

$$PIC = 1 - \sum_{i=1}^k P_i^2 - \sum_{i=1}^{k-1} \sum_{j=i+1}^k 2P_i^2 P_j^2$$

Where  $k$  is total alleles number,  $P_i$  and  $P_j$  are allele frequency of  $i^{th}$  and  $j^{th}$  allele respectively at microsatellite locus observed.

## RESULTS AND DISCUSSION

The developments of some genetic markers that are used in cattle breeding because of the molecular markers are more discriminative and accurate than their phenotypic characters. But, the utilizing of this marker has not been maximally yet. By using this marker would have helped in handling cattle breeding management system (Ge *et al.* 2002).

Breed specific allele information for Indonesian domesticated cattle until recently is limited. From previous studies (Muladno *et al.* 2000; Noor *et al.* 2000; Winaya 2000; Winaya *et al.* 2000; Mohamad *et al.* 2007; Ugla 2008) that use DNA microsatellite marker has been showed that these markers can also give description of breed specific allele and genetic relationship between Indonesian native cattle (i.e. Aceh, Pesisir, Madura, Bali, PO and PFH), but this result can not shows yet the description about the genetic variation of Indonesian native cattle based on Y-chromosome DNA microsatellite in more detail. So, this study may be consideration as a preliminary data base to determine the genetic composition of Indonesian native cattle in general. PCR product using seven primers which is flanks microsatellite sequence were loaded using polyacrilamide gel electrophoresis / PAGE 10% then continued with silver staining. Microsatellite allele was detected manually either its number and its size and next analysis.

Separation result using polyacrylamide gel generally can determine number and allele frequency as shown in Table 1 below.

Table 1 showed that we found only two alleles for all of locus by means 1.7, both in Aceh or Pesisir cattle. So, this allele still could not be determined as polymorphic allele yet, because according to FAO minimum standard locus has minimally four different alleles to be used as judgment in determining genetic differentiation between groups of cattle (Pandey *et al.* 2006). From this study it is also revealed that genetic composition of Aceh and Pesisir cattle are also contain of *B.indicus* genetic. Because INRA124 also the one of *B. indicus* locus (Hanotte *et al.* 2000; Edwards *et al.* 2007). These result, therefore, are in line with the previous research results that *B indicus* is one of genetic source of the genetic composition of Aceh and Pesisir cattle (Mohamad *et al.* 2007; Ugla 2008).

From previous study (Winaya *et al.* 2008), the highest heterozygosity ( $h$ ) value has been found in microsatellite locus of DYS 199 (68%) in Madura cattle population. It shows that the higher the number of allele, higher the heterozygosity tendency will be. Again, according to FAO guideline, here must be four different allele locus minimum to justify the differentiation or genetic variation between breed (Pandey *et al.* 2006).

From polymorphic information content ( $PIC$ ) value, the Y-chromosome microsatellite marker has reveals  $PIC$  value from 0.18 to 0.23. This result could be explained that in generally all of the locus could not be determined as an informative allele for population genetic analysis, because the  $PIC$  value less than 0.50 (Botstein *et al.* 1980). As Meadows *et al.* (2006) study has been found the low value in nucleotide variation sequence of specific Y-chromosome area in some animal species, including cattle. So, from this study we still need more locus of Y-chromosome microsatellite marker for genetic analysis of Indonesian native cattle in generally. As we know that until recently for genetic evaluation is trend to use the Y-chromosome microsatellite marker. So, eventhough this study was not found the polymorphic locus, but for future study we still need more number of Y-chromosome DNA microsatellite marker, because this marker could be as a tool to determine the genetic specific of Indonesian native cattle in much more detail.

Table 1. Number of cattle assayed, allele number and frequency of seven Y-chromosome microsatellite locus in population of Aceh and Pesisir cattle

LOCUS	ACEH			PESISIR		
	1	2	3	1	2	3
INRA 008	18	2	A = 3 (0.17) B = 15 (0.83)	15	2	A = 3 (0.20) B = 12 (0.80)
INRA 057	18	1	A = 18 (1)	15	2	A = 2 (0.13) B = 13 (0.87)
INRA 062	18	2	A = 6 (0.33) B = 12 (0.67)	15	2	A = 7 (0.47) B = 8 (0.53)
INRA 124	18	2	A = 5 (0.28) B = 13 (0.72)	15	2	A = 3 (0.20) B = 12 (0.80)
INRA 126	18	1	A = 18 (1)	15	2	A = 1 (0.07) B = 14 (0.93)
DYS 199	18	2	A = 3 (0.17) B = 15 (0.83)	15	1	A = 15 (1)
INRA 189	18	2	A = 4 (0.22) B = 14 (0.78)	15	1	A = 15 (1)

Note: 1) number of cattle assayed; 2) allele number; 3) allele frequency.

Table 2. Heterozygosity ( $h$ ) and Polymorphic Information Content ( $PIC$ ) value of Y-chromosome microsatellite allele between Aceh and Pesisir cattle population

LOCUS	ACEH		PESISIR	
	$h$	$PIC$	$h$	$PIC$
INRA 008	0.30	0.24	0.34	0.27
INRA 057	0	0	0.24	0.20
INRA 062	0.47	0.34	0.53	0.37
INRA 124	0.43	0.32	0.34	0.27
INRA 126	0	0	0.14	0.12
DYS 199	0.30	0.24	0	0
INRA 189	0.36	0.28	0	0
Mean $\pm$ SD	0.27 $\pm$ 0.19	0.20 $\pm$ 0.14	0.23 $\pm$ 0.20	0.18 $\pm$ 0.14

## CONCLUSIONS

The genetic flow of both *B taurus* and *B indicus* influencing genetic composition of Indonesian native cattle, including Aceh and Pesisir cattle, eventhough, we knew that Indonesian native cattle breed generally still have a lineage from Banteng (*Bos banteng*), the one of ancestor cattle in the world until nowadays. This trend has also been described in this study, by using Y-chromosome microsatellite markers, those cattle populations have indicated any genetic composition of *B indicus*, with locus INRA124 as reference.

The use of molecular marker of Y-chromosome microsatellite has generally described potentials and genetic variations of Aceh and Pesisir cattle, eventhough there was not found the polymorphic and informative allele. But, for the next study we can use more locus of Y-chromosome DNA microsatellite marker, because we still need more data base for genetic

potential of Indonesian native cattle, especially the genetic composition of Aceh and Pesisir cattle. So, the genetic existence of those cattle as specific cattle can be improved.

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