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IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS ON CATTLE BREEDS IN INDONESIA USING BOVINE 50K

Identifikasi Single Nucleotide Polymorphisms *pada Bangsa Sapi di Indonesia Menggunakan* Bovine 50K

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ABSTRACT

Single nucleotide polymorphisms (SNPs) abundant in bovine genome influence genetic variation in biological mechanism. The study aimed to identify SNPs on Indonesian cattle breeds and analyze their genetic diversity using Bovine 50K SNP chip. Twenty eight "Ongole Grade" (OG) beef cattle and 20 "Holstein Friesian" (HF) dairy cattle were used for the Infinium II assay test. This assay included amplification of genomic DNA, fragmentation, precipitation, resuspension, hybridization, processing bead chip for single-base extension, and imaging at iScan. Data and clusters were analyzed using GenomeStudio software. The Bovine 50K SNP chip containing 54,609 SNPs was observed spanning all chromosomes of bovine genome. Genotyping for the total SNPs was successfull based on Call Rate, GeneCall and GeneTrain scores. Most SNP markers had alleles that shared among the individuals or breeds, or had specific alleles at distinctive frequencies. Minor allele frequency (MAF) spreads equally with intervals of 0-0.5. The breeds of OG and HF tended to be separated in different clusters without considering their genetic history and twin or normal. This result suggests that most individuals are closely related to one another, regardless of the same breed. Some genes identified on chromosomes 3, 4, 5, 7, 13, 17 and 18 were located in the loci/regions that contained SNPs with specific alleles of either HF or OG breed. These SNPs were more powerful for differentiation of beef cattle and dairy cattle than among individuals in the same breed. These SNP variations and genetic relatedness among individuals and breeds serve basic information for cattle breeding in Indonesia.

[***Keywords*:** Beef cattle, dairy cattle, Bovine 50K, SNP, Indonesia]

ABSTRAK

Single nucleotide polymorphisms *(SNPs) yang melimpah dalam genom sapi berpengaruh terhadap variasi genetik dalam mekanisme biologi. Penelitian ini bertujuan untuk mengidentifikasi SNP pada sapi di Indonesia dan menganalisis keragaman genetiknya menggunakan* Bovine 50K SNP chip. *Dua puluh delapan ekor sapi potong Peranakan Ongole (PO) dan 20 ekor sapi perah Friesian Holstein (FH) digunakan untuk uji Infinium II menggunakan* Bovine 50K SNP chip. *Proses analisis meliputi amplifikasi DNA genom, fragmentasi, presipitasi, resuspensi, hibridisasi, pemrosesan* bead chip *untuk reaksi* single-base extension*, dan* imaging *pada iScan. Data dan klaster dianalisis menggunakan perangkat lunak GenomeStudio.* Chip *yang mengandung 54.609 SNP diobservasi yang mencakup semua kromosom pada genom sapi. Analisis* genotyping *total SNP berdasarkan* Call Rate*,* GeneCall*, dan skor* GeneTrain *menunjukkan keberhasilan* genotyping. *Mayoritas marka SNP memiliki alel yang umum di antara individu atau bangsa sapi, atau memiliki alel spesifik dengan frekuensi yang berbeda. Nilai* minor allele frequency *(*MAF*) tersebar merata pada selang nilai 0-0,5. Sapi potong PO dan sapi perah FH terpisah dengan jelas dalam klaster yang berbeda tanpa mempertimbangkan sejarah genetiknya maupun kembar atau normal. Hasil ini menunjukkan kedekatan antarindividu karena kesamaan bangsa sapi. Beberapa gen yang diidentifikasi pada kromosom 3, 4, 5, 7, 13, 17, dan 18 terletak dalam lokus/daerah yang mengandung SNP dengan alel spesifik pada sapi FH atau PO, SNP tersebut lebih berguna untuk membedakan antara sapi potong dan sapi perah, daripada antara individu dalam rumpun yang sama. Variasi SNP dan keterkaitan genetik antarindividu dan bangsa sapi tersebut bermanfaat sebagai informasi dasar dalam pemuliaan sapi di Indonesia.*

[***Kata kunci*:** Sapi potong, sapi perah, *Bovine 50K*, SNP, Indonesia]

INTRODUCTION

Referring to the category of cattle worldwide, beef cattle and dairy cattle are the most cattle farms existing in Indonesia. Beef cattle are great important in Indonesia because of their economic and socio-cultural values. In addition to beef cattle, dairy cattle farms also have a potential role in increasing farmers' income and livestock development. In addition to meat and milk, both cattle breeds produce organic fertilizer and increase the use of agricultural waste biomass (Thohari 2000; Aryogi and Romjali 2009). Thus, cattle development has a strategic value to achieve food security in parallel with growing population in Indonesia.

The diverse geographical areas with different climate, environmental conditions and local socio-culture influence high diversity of cattle germplasm with distinctive morpho-physiological characteristics. The high diversity of cattle is useful for farms development since the genetic materials are needed to develop new breed with high productivity and other interest characters (Diwyanto 2005).

Cattle "Ongole Grade" (OG) belonging to species *Bos indicus* and sub-family Bovinae are commonly found in Java and other regions in Indonesia. This OG is relatively pure its genetic, but has experienced adaptation in Indonesia. This cattle breed is highly desirable by farmers as it is profitable, and easy and low cost in maintenance. While "Holstein Friesian" (HF), a dairy cattle breed from *Bos taurus* originated from Holland, has a high productivity of milk. HF could be used as genetic material in dairy cattle breeding program to increase milk production (Aryogi and Romjali 2009; Prahanisa *et al*. 2011). Considerable potency of cattle population leads to investigation of relatedness and genetic diversity using molecular characterization as basic information for future breeding program.

Molecular markers in cattle have been applied on several target characters. The characters are not only related to growth and productivity of meat and milk, but also disease resistance, fertility and environmental stress tolerance (Singh *et al*. 2014). On the basis of detection techniques, molecular markers are categorized into hybridization-based and PCR-based markers in cattle genetic research. The PCR-based markers are divided into sequenced-targeted PCR assay and arbitrary PCR assay. The former categories include cleaved amplified polymorphic sequence, alleles specific PCR, PCR amplification of specific alleles, simple sequence length polymorphism, and sequence-targeted microsatellite site. And, arbitrary PCR assays are such as RAPD and microsatellite-primed PCR. Microsatellites/simple sequence repeat (SSR) markers are popular in genetic characterization on cattle due to their easy application and high variation (Sunnucks 2001; Deb *et al*. 2013).

Single nucleotide polymorphisms (SNPs), a bi-allelic type of marker, become popular because of many advantages. Compared to other genomic variation, SNPs are the most abundant known so far in animal. The SNPs could be a potential genetic marker and get a higher interest because of the stability and high-throughput automated analysis (Fries *et al*. 1990; Heaton *et al*. 2002). To complement the development of molecular markers on the basis of single or few loci, high throughput genotyping via next generation sequencing (NGS) in the form of array or chip-based markers is more useful. Such markers could be used for a variety of purposes including genome-wide association studies, population studies, bulk segregant analyses, quantitative trait loci (QTL) interval mapping, whole genome profiling, background screening, etc. (Kim *et al*. 2006; Wenzl *et al.* 2007; Gupta *et al*. 2008).

In cattle, genomic evaluation was initiated and available years ago. The first generation bead chip with low density, Bovine 3K bead chip, was introduced to increase the adoption of genomic testing in 2010 (Illumina Inc. 2011a; Wiggans *et al*. 2011). In addition, the high density bead chip called as Bovine 50K SNP from Illumina was commercialized. Unlike Bovine 3K, Bovine 50K SNP chip (Infinium) was available with more than 50,000 informative SNPs that uniformly span the entire bovine genome. Rapid detection of Bovine 50K chip was evidenced by the number of new individuals tested (Illumina Inc. 2011b;Wiggans *et al*. 2011).

The Bovine 50K chip has been used for many studies and assists selection in cattle breeding program in other countries. However, so far, no study reported bovine genomic evaluation in Indonesian cattle population/breed using the high throughput technology. This study aimed to identify SNP on cattle breeds in Indonesia and to analyze their genetic diversity using Bovine 50K SNP chip with iScan.

MATERIALS AND METHODS

Individual Materials

A total of 48 individuals comprising of 28 beef cattle (Ongole Grade/OG) and 20 dairy cattle (Holstein Friesian/HF) were used in this study. The OG and HF

cattle breeds were obtained from the collection of Beef Cattle Research Station (BCRS) and Indonesian Research Institute for Animal Production (IRIAP), respectively. Both the research institutes are under the Indonesian Agency for Agricultural Research and Development (IAARD). Most individuals were female accounting for 97.9% of total. According to historical aspect but not genetically, most of the cattle used were considered as twinning, and only 13 individuals were being as normal cattle for comparison of analysis. The age of cattle ranged diversely including calf, heifer and mother cows. In this study, all individuals were fed and maintained following the standard recommendation management. The list of all individuals of cattle along with the detailed information is presented in Table 1.

Isolation and Concentrating of DNA

For DNA isolation, cattle blood was collected using sterile needles and syringe, and then put in a 10 ml specific tube. The blood was kept in ethanol and stored in freezer (-80°C) until used. DNA isolation was done with QIAmp DNA blood mini kit (Qiagen) following the protocol from the biotechnological company. The DNA was eluted with TE buffer and migrated on 0.8% agarose gel electrophoresis. DNA concentration and purity were estimated by measuring the absorbance at 260/280 and 260/230 using NanoDrop1000. The DNA concentration was adjusted to 50 ng μl^{-1} as recommended for iScan analysis by concentrating it with SpeedVac (Thermoscientific). The pure genomic DNA was stored and prepared at least 15 µl to meet the requirement for Infinium II assay.

SNP Genotyping Using Illumina Bovine 50K SNP Chip

All cattle breeds were genome-wide genotyped with Infinium II assay using Bovine 50K SNP chip (Illumina Inc., San Diego) which comprises SNPs covering the bovine genome (Matukumalli *et al.* 2009; VanRaden *et al.* 2009). Approximately 200 ng of genomic DNA of each individual was used for the assay and samples were processed according to the Illumina Infinium-II assay manual. Briefly, each sample was whole-genome amplified, fragmented, precipitated and re-suspended in an appropriate hybridization buffer. Denatured samples were hybridized on the prepared BovineSNP50 chip for a minimum of 16 hours at 48°C. Finally, the bead chips were processed for the single-base extension reaction, stained and imaged on an Illumina iScan array.

Normalized bead intensity data for each sample were loaded into the GenomeStudio V2009.1 software facilitated by Illumina, which converted fluorescent intensities into SNP genotypes. SNP clusters for genotype calling were examined for all SNPs. SNP was identified based on the following criteria: (1) the number of genotype group, i.e. one or none (e.g. only AA genotype and no AB or BB), (2) the minor allele frequency (MAF), and (3) proportion of genotyped individuals based on Call Rate, GeneTrain score cutoff of 0.25 and 50% GeneCall (GC50) applied to the whole dataset. Thus, the overall genotyping reliability for the total SNPs was assessed by estimating SNP counts above conventionally used threshold and average values for Call Rate, GC50 and GeneTrain scores. These measures provide some general information about quality and performance of SNPs (Illumina Inc. 2011a; Grattapaglia *et al*. 2011). Clustering heat map and related SNP analyses were performed with GenomeStudio. The heatmap was generated based on euclidean distance, of which the variables measure were analyzed automatically for clustering.

RESULTS AND DISCUSSION

Performance and Quality of SNP

Genome-wide genotyping results from 54,609 SNPs in the Bovine 50K array revealed the data output generated with the Illumina GenomeStudio software with a no call threshold of 0.25. The performance of call rate, GeneCall (GC50) and GenTrain of SNPs is presented in Figure 1. A Call Rate is defined as the fraction of called SNPs per sample over the total number of SNPs in the dataset with a standard quality threshold of 95%. The Call Rate indicated a high quality of the identified SNP as demonstrated that proportion of SNPs with Call Rate of > 95% was 81.25%. The proportion of SNPs with 50% GeneCall (GC50) scores of > 0.40 was around 98.5% (Fig. 1A) with an average of 0.818. GenTrain score of SNP representing cluster separation was the lowest at 0.35, higher than the recommended threshold (Illumina Inc. 2011a; 2011b; Hoffman *et al*. 2012) (Fig. 1B). As supported by previous study, GenTrain score as low as 0.3 can still be successfully used to determine a degree of cluster separation (Yan *et al*. 2010). Above 50% of the SNPs screened in this study

Table 1. List of individuals of cattle coordinated by research institutes under IAARD for Infinium II assay of Bovine 50K bead chip using iScan array.

Sample code[1)]	Sex	History	Breed type	Collection
X-A-KB0-PR-IK3-1	Female	Historical twinning (triplet calf of IK3)	Dairy cattle	IRIAP (Ciawi farm)
X-D-KB0-PR-B758	Female	Historical twinning	Dairy cattle	IRIAP (Cicadas farm)
X-D-KT2-PR-2321	Female	Normal	Dairy cattle	IRIAP (Ciawi farm)
X-D-KB0-PR-A755	Female	Historical twinning	Dairy cattle	IRIAP (Cicadas farm)
X-A-KB0-PR-IK3-2	Female	Historical twinning (triplet calf of IK3)	Dairy cattle	IRIAP (Ciawi farm)
X-D-KB0-PR-B754	Female	Historical twinning	Dairy cattle	IRIAP (Cicadas farm)
X-D-KT2-PR-2470	Female	Normal	Dairy cattle	IRIAP (Ciawi farm)
X-D-KB0-PR-A752	Female	Historical twinning	Dairy cattle	IRIAP (Cicadas farm)
X-A-KB0-PR-IK3-3	Female	Historical twinning (triplet calf of IK3)	Dairy cattle	IRIAP (Ciawi farm)
X-D-KB0-PR-B760	Female	Historical twinning	Dairy cattle	IRIAP (Cicadas farm)
X-D-KT2-PR-2308	Female	Normal	Dairy cattle	IRIAP (Ciawi farm)
X-A-KB0-PT-09/38	Female	Historical twinning	Beef cattle	BCRS (Grati farm)
X-I-KB1-PR-IK3	Female	Mother cow (genetical twinning with triplet calves)	Dairy cattle	IRIAP (Ciawi farm)
X-D-KB0-PR-A759	Female	Historical twinning	Dairy cattle	IRIAP (Cicadas farm)
X-D-KT2-PR-2126	Female	Normal	Dairy cattle	IRIAP (Ciawi farm)
X-I-KB0-PT-9702	Female	Historical twinning	Beef cattle	BCRS (Grati farm)
X-D-KT2-PR-2522	Female	Normal	Dairy cattle	IRIAP (Ciawi farm)
X-D-KB0-PR-A753	Female	Historical twinning	Dairy cattle	IRIAP (Cicadas farm)
X-D-KB0-PR-B756	Female	Historical twinning	Dairy cattle	IRIAP (Cicadas farm)
X-U-KT2-PT-09931	Female	Normal	Beef cattle	BCRS (Grati farm)
X-D-KT2-PR-1215	Female	Normal	Dairy cattle	IRIAP (Ciawi farm)
X-D-KB0-PR-A751	Female	Historical twinning	Dairy cattle	IRIAP (Cicadas farm)
X-D-KB0-PR-B757	Female	Historical twinning	Dairy cattle	IRIAP (Cicadas farm)
X-U-KT2-PT-1960	Female	Normal	Beef cattle	BCRS (Grati farm)
X-U-KB0-PT-R09737	Female	Historical twinning	Beef cattle	BCRS (Grati farm)
X-U-KB0-PT-R09712	Female	Historical twinning	Beef cattle	BCRS (Grati farm)
Y-A-KB0-PT-09/29	Male	Historical twinning	Beef cattle	BCRS (Grati farm)
X-I-KB0-PT-R07492	Female	Historical twinning	Beef cattle	BCRS (Grati farm)
X-U-KT2-PT-07427	Female	Normal	Beef cattle	BCRS (Grati farm)
X-A-KB0-PT-7418	Female	Historical twinning	Beef cattle	BCRS (Grati farm)
X-I-KB0-PT-R09889	Female	Historical twinning	Beef cattle	BCRS (Grati farm)
X-U-KB0-PT-R09738	Female	Historical twinning	Beef cattle	BCRS (Grati farm)
X-A-KB0-PT-K09814	Female	Historical twinning	Beef cattle	BCRS (Grati farm)
X-U-KT2-PT-09771	Female	Normal	Beef cattle	BCRS (Grati farm)
X-A-KB0-PT-06/11	Female	Historical twinning	Beef cattle	BCRS (Grati farm)
X-A-KB0-PT-05/04	Female	Historical twinning	Beef cattle	BCRS (Grati farm)
X-U-KT2-PT-09808	Female	Normal	Beef cattle	BCRS (Grati farm)
X-A-KB0-PT-R07514	Female	Historical twinning	Beef cattle	BCRS (Grati farm)
X-U-KT2-PT-09982	Female	Normal	Beef cattle	BCRS (Grati farm)
X-I-KB0-PT-K07409	Female	Historical twinning	Beef cattle	BCRS (Grati farm)
X-A-KB0-PT-07616	Female	Historical twinning	Beef cattle	BCRS (Grati farm)
X-U-KB0-PT-9766B	Female	Historical twinning	Beef cattle	BCRS (Grati farm)
X-I-KB1-PT-7415	Female	Genetical twinning	Beef cattle	BCRS (Grati farm)
X-U-KB0-PT-9766A	Female	Historical twinning	Beef cattle	BCRS (Grati farm)
X-I-KB0-PT-R09728	Female	Historical twinning	Beef cattle	BCRS (Grati farm)
X-A-KB0-PT-06/12	Female	Historical twinning	Beef cattle	BCRS (Grati farm)
X-U-KB0-PT-09/01	Female	Historical twinning	Beef cattle	BCRS (Grati farm)
X-U-KT2-PT-09981	Female	Normal	Beef cattle	BCRS (Grati farm)

[1)]Description of sample code: Sex (X = female, Y = male); Cattle type according to age (A = calf, I = mother cow, D = heifer, U = unidentified as mother/calf/heifer); Cattle type according to heredity twinning (KB0 = historical twinning, KB1 = genetical twinning, KT2 = normal cow); Cattle type according to breed (PR = dairy cattle, Holstein Friesian/HF; PT = beef cattle, Ongole Grade/OG).

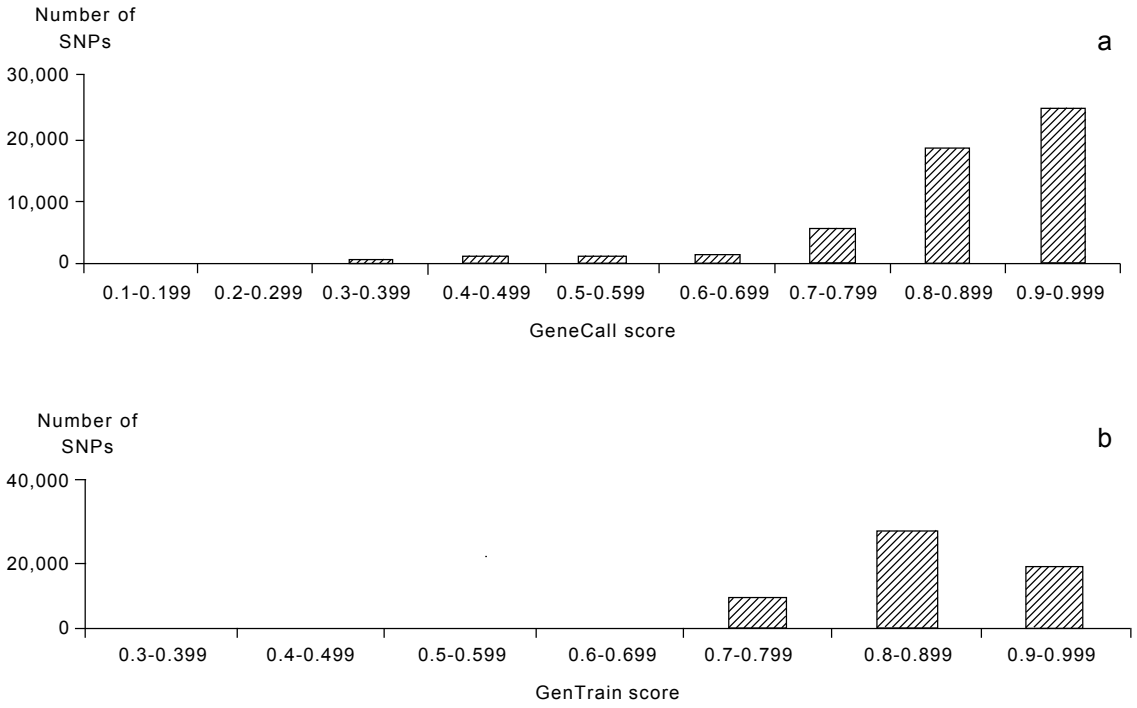

Fig.1. Distribution of SNPs across classes observed in 48 individuals of cattle using Infinium assay with Bovine 50K bead chip; (a) GeneCall50, (b) GenTrain.

possessed GC50 and GenTrain scores near one and can be considered as sufficient quality to be correctly scored by the Illumina GenomeStudio genotyping software without manual intervention. The overall parameters for the 54,609 SNPs demonstrated the success of genotyping reliability of total cattle observed in this study.

SNP Distribution and Allele Frequency

The SNP existed in the Bovine 50K developed by Illumina showed their even distribution across 60 chromosomes of entire bovine genome, 29 pair of autosomes and one pair of sex chromosomes (X and Y). The number of SNPs per chromosome ranged from one (on chromosome Y) to almost 3,500 SNPs on chromosome 1 (Fig.2A). Tyler-Smith (2008) reported that as in other mammals, males have an X and a Y chromosome and females have 2x chromosomes, thus, only the autosome was used in this study. The total SNPs were found to have homology with several regions in bovine genome such as BTA, BTB, ARS-BFGL-NGS, UA-IFASA, and Hapmap-SCAFFOLD.

An even distribution of MAF was observed (Table 2) with rive continued classes from 0 to 0.5. A relatively similar number of SNPs was found in MAF class of 0.3-0.399 (15.54%) and 0.4-0.5 (13.92%). The highest number of SNPs possessed MAF of less than 0.199 was 31.56% (17,236/54,609). Selected SNP markers with high MAF scores in this study could have a high impact and useful on genetic diversity analysis, given the great differentiating power that is in good agreement with previous study (Yan *et al*. 2010). The difference in allele frequencies may be attributable to divergence of the cattle breeds (Matukumalli *et al*. 2009; Dadi *et al*. 2012). In addition, information on the allelic frequencies of these SNPs should help determine the usefulness of this marker for analysis of other cattle breeds in Indonesia.

Particular emphasis was placed on SNP polymorphism, of which for homozygous, one SNP (for example G/C) was able to produce two alleles (G and C). For 54,609 SNPs (with just one SNP per locus), a maximum total of 109,218 alleles can be detected. Of number SNPs surveyed, alleles of A/G (22,579/54,609 or 41%) seemed predominantly in the population and followed with T/C (34%). Alleles of A/C and T/G had a relatively similar proportion, accounting for 10% and 9%, respectively (Fig. 2B). While A/T and T/A were identified as minor alleles in the total of 48 individuals. Clearly, most SNP markers had alleles which were shared among the individuals and/or

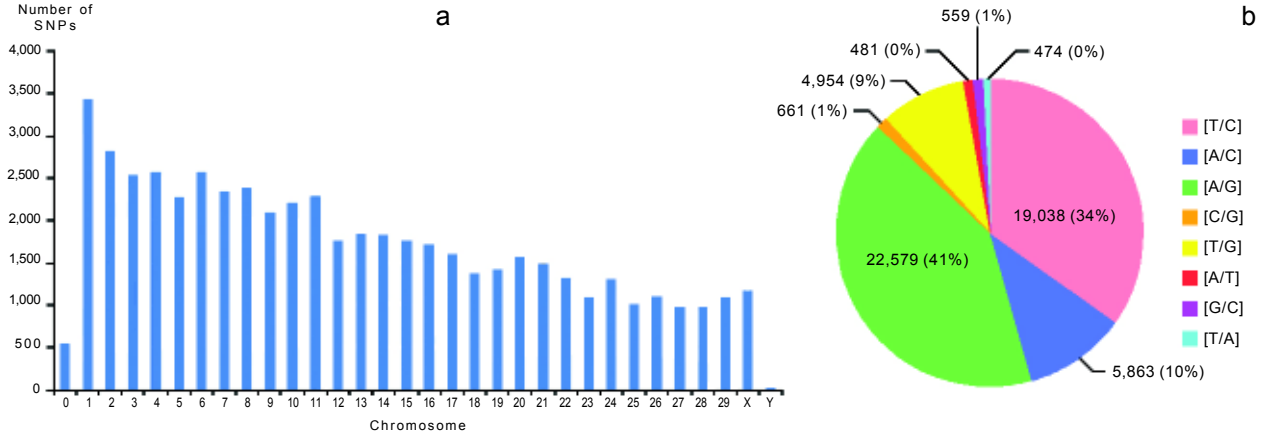

Fig.2. Distribution and proportion of SNPs for Infinium assay observed on cattle breeds in Indonesia; (a) Distribution of SNPs spanning on chromosome in Bovine 50K bead chip, (b) Proportion of SNPs observed in 48 individuals according to Bovine 50K.

Table 2. SNPs with minor allele frequency (MAF) of 5% detected in 48 individuals of cattle.

MAF range	Number of SNPs	Percentage (%)
0.0-0.099	17,236	31.56
0.1-0.199	11,343	20.77
0.2-0.299	9,937	18.20
0.3-0.399	8,490	15.54
0.4-0.499	7,603	13.92

breeds, or had specific alleles at distinctive frequencies as demonstrated in this study. The major alleles produced by some markers could be specific in Indonesian cattle, leading allelic deviation in the breeds. Major alleles were also essentially equivalent to minor allelic frequency (MAF) in information content for differentiation of animals (Kruglyak 1997; Hasegawa *et al*. 2014). All SNPs common to both breeds probably arose before the divergence of the breeds. Importantly, the rare and minor alleles could influence economically important traits in livestock species (Freking *et al*. 2002; Smit *et al*. 2003).

Analysis of Cluster and Genetic Diversity of Cattle Breeds

Scoring of SNP among individuals using GenomeStudio generally produced three clusters denoting the AA homozygote, BB homozygote and AB heterozygote, but some of data dots ambiguously appeared between the clusters in the genoplot as depicted in Figure 3. For examples, SNP in ARS-BFGL-NGS-18937 revealed AA genotype for a total of 48 individuals (Fig. 3A), in contrast, ARS-BFGL-NGS-10077 showed mostly BB genotype (Fig. 3B). While Hapmap 27796-BTA-21954 resulted three clusters which presented AA genotype (28 individuals), AB (14 individuals) and BB (6 individuals) (Fig. 3C). In respect to some SNPs showing only homozygote, they were predominated by BB genotype accounting the frequency of 0.458 and AA with 0.314 value in total of individuals observed. A few individuals contained heterozygotes with proportion of 0.228. This cluster separation as denoted by GenTrain score could explain the three classes's separation (AA, AB and BB). In addition to represent SNP quality, the reliable classes' pattern of the cattle breeds virtually reflected their genetic nature based on the stringent SNPs existing in Bovine 50K array. This powerful SNPs in this study is in good agreement with previous studies on BovineSNP50 Bead Chip for genotyping various breeds and species in the tribe *Bovini* (Bae *et al*. 2010; Michelizzi *et al*. 2011; Dadi *et al*. 2012).

Genetic variation within or among breeds is usually explained in terms of allel frequencies. Figure 4 depicts heat map of the 48 individuals according to Bovine 50K SNP. Two main clades were generated and showed almost clear separation of different breeds, 20 individuals mostly HF (with exception of three OG namely X_A_KBO_PT_09/38, X_U_KT2_PT_09931 and X_I_KBO_PT_9702) in clade I and 28 individuals of OG belonging to the clade II. A few HF individuals, i.e. X_D_KBO_PR_B757, X_D_KT2_PR_1215, and X_D_KBO_PR_A751 that were preferentially grouped with most OG (clade II) demonstrated their close

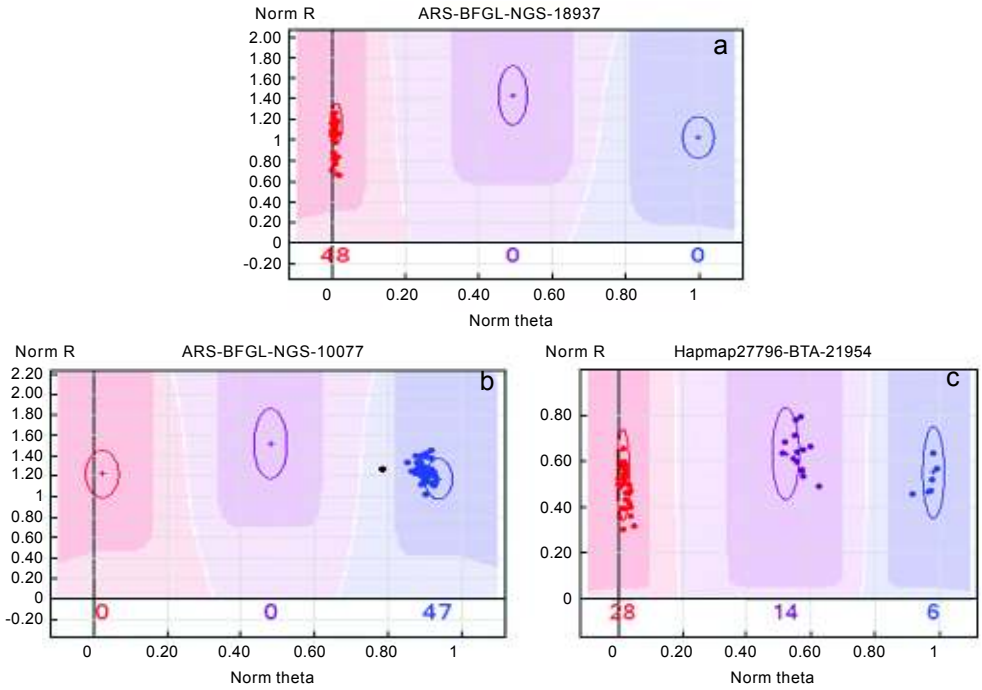

Fig. 3. Examples of SNP genotyping clusters on 48 individuals of cattle observed based on Bovine 50K bead chip using BeadStudio software. (a) Homozygote allele AA, (b) homozygote allele BB, and (c) heterozygote allele AB.

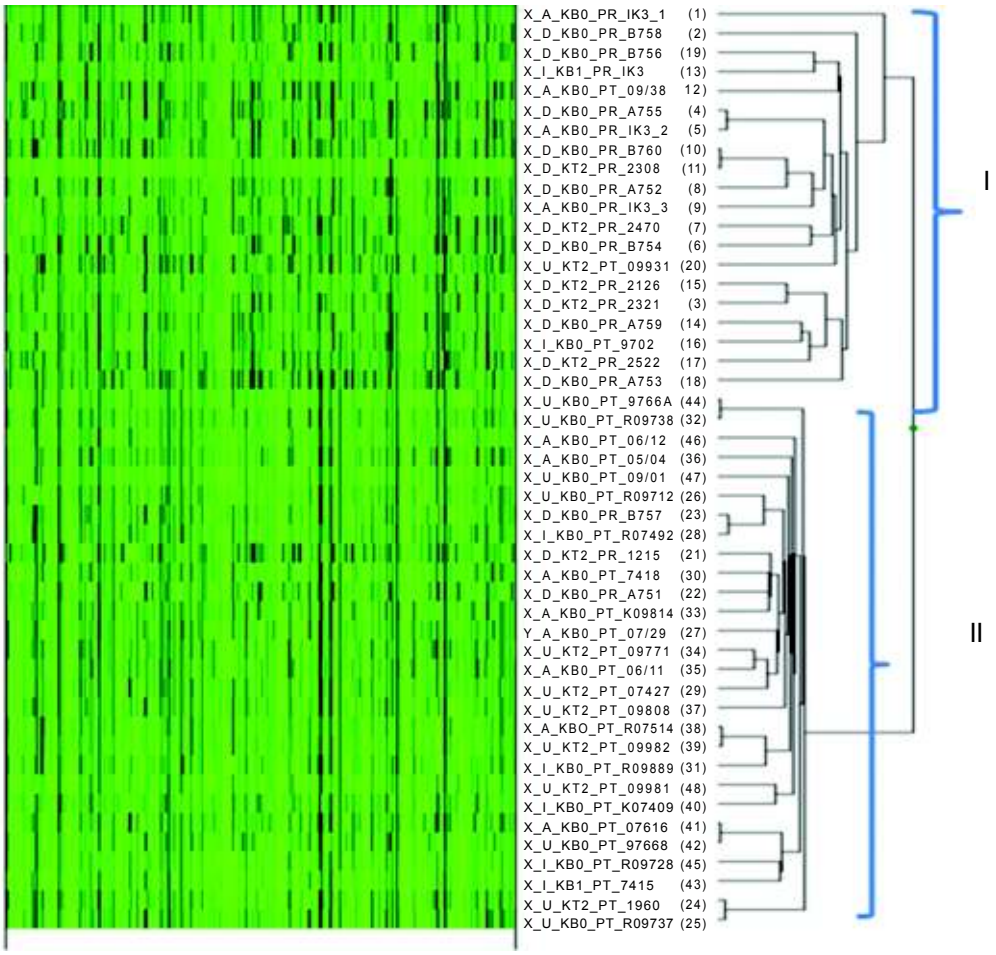

Fig. 4. Cluster of 48 individuals of cattle based on Bovine 50K SNP chip generated with GenomeStudio; I = Mostly Ongole Grade (OG) breed, II = Mostly Holstein Friesian (HF) breed.

relationship compared to other individual dairy cattle. The only one genetical twinning for each OG (X_I_KB1_PT_7415) and HF (X_I_KB1_PR_IK3) grouped in different clades, reflecting that the two had far genetic distance. Another interesting example, these SNPs were able to identify the genetic twinning mother cow (X_I_KB1_PR_IK3) and her triplet calf (IK3_1, IK3_2 and IK3_3) in the same clade (clade I). Parent-child heritability frequency would confirm the parent-child relationship (Bae *et al.* 2008; 2010). Thus, OG beef cattle and HF dairy cattle generally tended to be clearly separated in different clusters without considering their genetic history, sex, historical twin and normal. This result indicated that most individuals were closely related to one another, regardless of the same breed. However, no clear differentiation of individuals found within breed either in OG or HF, indicating that the SNPs developed based on dairy cattle genome (Bovine 50K SNP) were only useful to differentiate cattle according to the genetic background of individual within breed. This is consistent with the preliminary analysis in previous report (Lestari and Tasma 2012). Moreover, these results demonstrated that inbreeding and selection had little effect on reducing genetic diversity and differentiating both within HF and OG breeds in Indonesia at a genome-wide level, similarly to the study case of other HF breed in Australia (Zenger *et al*. 2007). These SNP markers could be useful for association analysis with phenotypic characters of cattle such as meat productivity, beef quality and milk quality. In line with the previous report (Bae *et al*. 2010), further research could examine the genetic effects of the SNPs on various economic characters on cattle.

When the location on a chromosome with copy number variation in *Bos taurus* (Bae *et al*. 2010) was overlapped with the regions/loci containing SNP in our study, some genes were identified on chromosomes 3, 4, 5, 7, 13, 17 and 18 whose positions were in the loci we observed in this study (Fig. 5). For example, chromosome 3 at position of 36,163,190-36,

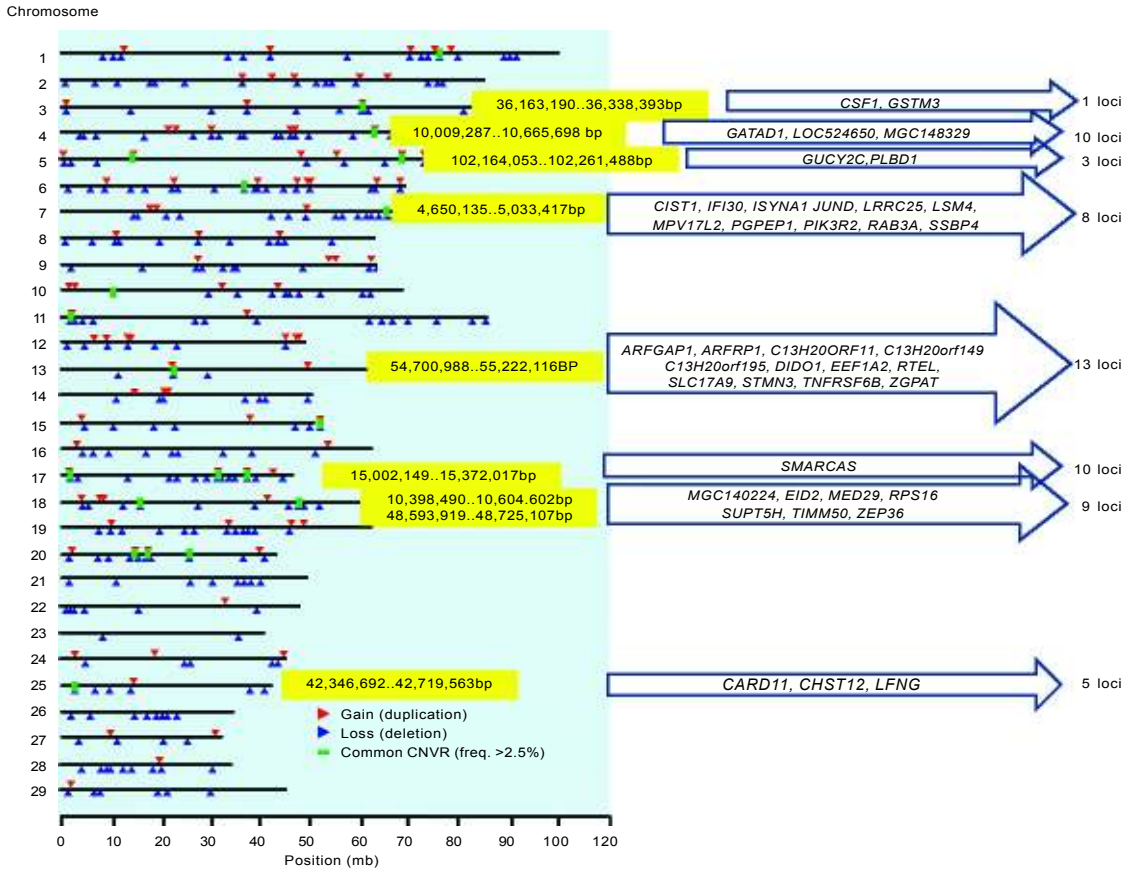

Fig. 5. Map of identified locations of copy number variations in *Bos taurus coreanae* in relation with bovine genome regions of Bovine 50K corresponding to known genes used to observe SNP among 48 individuals of cattle. From left to right: first box with blue shade is map of identified copy number variation in the genome of *Bos taurus* (Bae *et al*. 2010), yellow-shaded box is selected regions in chromosomes identified by Bae *et al.* (2010) which have overlap position with SNP observed in our study, arrows contain genes in cattle coincident with the selected regions consisting of SNP in our study (the right ones).

338 and 393bp showed overlap with one region (ARS-BFGL-NGS-66 946) which had two genes (CSF1 and GSTM3). Interestingly, on chromosome 13, there were 12 genes (ARFGAP1, ARFRP1, C13H20ORF11, C13H20orf149, C13H20orf195, DIDO1, EEF1A2, RTEL, SLC17A9, STMN3, TNFRSF6B, ZGPAT) that were coincident with the positions of 13 loci (ARS-BFGL-NGS-42 070, 16 884 smear-no-rs, Hapmap30591-BTA-159 623, ARS-BFGL-NGS-76 148, ARS-BFGL-NGS-1120, ARS-BFGL -NGS-118 051, ARS-BFGL-BAC-12 577, ARS-BFGL-BAC-12 578, ARS-BFGL-NGS-85 574, ARS-BFGL-NGS-100 973, BTB-00,529,185, ARS-BFGL-NGS-25 461 and ARS -BFGL-NGS-82 625) in our study.

Notably, a total of 59 selected SNPs in our study that correspond to genes as identified previously (Bae *et al.* 2010) revealed specific alleles on OG and HF (Table 3). These reference and alternate alleles were detected on selected chromosomes (3, 4, 5, 7, 13, 17

Table 3. List of selected SNPs located on gene regions identified in the beef and dairy cattle breeds.

SNP name	Chromosome	Position in bovine genome (bp)	SNP		Corresponding genes
			Dairy cattle (HF)	Beef cattle (OG)	
ARS-BFGL-NGS-66946	3	3677073	T	C	*CSF1,GSTM3*
ARS-BFGL-NGS-14645	4	10148342	T	C	*GATAD1, LOC524650, MGC148329*
Hapmap46397-BTA-105989	4	10172043	T	C	
BTB-01637746	4	10235907	T	C	
BTB-01538878	4	10261371	T	C	
Hapmap41620-BTA-70804	4	10324170	A	G	
Hapmap41484-BTA-22365	4	10450547	A	C	
BTB-02028475	4	10499057	A	C	
BTB-01238565	4	10528978	T	C	
BTB-00190485	4	10608965	A	G	
BTB-00172924	4	10648384	A	G	
ARS-BFGL-NGS-90522	5	102174236	A	C	*GUCY2C, PLBD1*
BTA-15444-no-rs	5	102253486	T	A	
Hapmap3063-BTA-15439	5	102308562	A	C	
BTB-00291042	7	4655753	T	C	*CIST1, IFI30, ISYNA1, JUND, LRRC25, LSM4, MPV17L2, PGPEP1, PIK3R2, RAB3A, SSBP4*
BTB-00290974	7	4729265	T	C	
ARS-BFGL-NGS-98087	7	4798483	T	C	
ARS-BFGL-NGS-107429	7	4835132	A	G	
ARS-BFGL-NGS-58779	7	4916633	A	G	
BTB-00292673	7	4953801	A	G	
Hapmap57767-ss46527024	7	5001007	A	G	
ARS-BFGL-NGS-110900	7	5027447	A	G	
ARS-BFGL-NGS-42070	13	54742445	T	C	*ARFGAP1, ARFRP1, C13H20ORF11, C13H20orf149, C13H20orf195, DIDO1, EEF1A2,RTEL, SLC17A9, STMN3, TNFRSF6B, ZGPAT*
BTA-16884-no-rs	13	54763115	A	G	
Hapmap30591-BTA-159623	13	54804053	T	C	
ARS-BFGL-NGS-76148	13	54829615	T	G	
ARS-BFGL-NGS-1120	13	54865583	T	C	
ARS-BFGL-NGS-118051	13	54895475	A	G	
ARS-BFGL-BAC-12577	13	54956566	A	C	
ARS-BFGL-BAC-12578	13	55006836	A	G	
ARS-BFGL-NGS-85574	13	55052389	A	C	
ARS-BFGL-NGS-100973	13	55090558	A	G	
BTB-00529185	13	55131886	A	C	
ARS-BFGL-NGS-25461	13	55183375	A	G	
ARS-BFGL-NGS-82625	13	55218560	T	G	

Table 3. (continued).

SNP name	Chromosome	Position in bovine genome (bp)	SNP		Corresponding genes
			Dairy cattle (HF)	Beef cattle (OG)	
BTB-01963792	17	15020783	A	C	*SMARCA5*
BTB-00673952	17	15052590	A	C	
ARS-BFGL-NGS-16708	17	15107947	T	G	
ARS-BFGL-NGS-26864	17	15127358	T	C	
ARS-BFGL-NGS-87957	17	15175882	A	G	
ARS-BFGL-NGS-32257	17	15211563	C	G	
ARS-BFGL-NGS-113029	17	15247955	A	C	
BTA-106183-no-rs	17	15271899	A	C	
BTA-106195-no-rs	17	15298798	T	C	
BTA-05721-rs29019877	17	15340860	T	C	
Hapmap30220-BTA-132038	18	10413454	A	G	*MGC140224*
ARS-BFGL-NGS-86596	18	10451525	T	G	
ARS-BFGL-NGS-14442	18	10491005	A	G	
ARS-BFGL-NGS-4535	18	10523212	A	G	
ARS-BFGL-NGS-41026	18	10556123	T	G	
Hapmap35421-SCAFFOLD98325_1119	18	48616951	C	G	*EID2, MED29, RPS16, SUPT5H, TIMM50, ZFP36*
ARS-BFGL-NGS-77973	18	48644344	A	G	
ARS-BFGL-NGS-42271	18	48678801	A	C	
ARS-BFGL-NGS-29923	18	48719962	T	C	
ARS-BFGL-NGS-101981	25	42364359	A	G	*CARD11, CHST12, LFNG*
ARS-BFGL-NGS-12443	25	42609054	T	C	
ARS-BFGL-NGS-116071	25	42631146	T	C	
ARS-BFGL-NGS-34717	25	42640462	T	C	
ARS-BFGL-NGS-30953	25	42687812	A	G	

and 18) in dairy and beef cattle, respectively. The point mutation existing in the cattle breeds in Indonesia varied with bi-allele of T/C, A/C, A/G, T/A, T/G and C/G. These base substitution which may affect phenotypic variation in different breeds of cattle may need to be further investigated and could provide insight into enrichment of phenotypic impact through genomic resources (Gan *et al*. 2008; Liu *et al*. 2008).

Information on genetic variation of cattle breeds in Indonesia based on bovine genome could complement and enrich previous studies. A number of researches in genome-wide SNP genotyping has been progressively achieved, such as cost-effective dairy cattle breeding programs (Hayes *et al*.2009), useful information on genetic variation of Korean Hanwoo breed (Dadi *et al*. 2012) and indicine and African cattle breeds (Matukumalli *et al*. 2009), and genome wide association for milk production in Danish Jersey cattle (May *et al*. 2010). Specific SNPs associated with genes have also been elucidated their association with targeted traits in dairy and beef cattle (Liu *et al*. 2011; Lu *et al*. 2011; Deb *et al*. 2014). Thus, our study is relevant with previous studies using Bovine SNP array conducted in many countries to offer a useful knowledge and promise for improving targeted traits in cattle.

Indeed, this result could be a good clue that the use of SNP chip is more powerful and could be functional in genetic diversity analysis. Several SNPs within and close to genes may provide an excellent solution to the disadvantage of SNP markers that have been used in diversity analyses (Zimin *et al*. 2009; Snelling *et al*. 2010). The SNP data represent a vast and largely untapped resource to assist the investigation of genetic studies in cattle, and also useful for cattle genetic improvement programs. The patterns of allele frequency variability observed among the breeds signal the genetic imprint of past and presumably on going episodes of selection (Hayes *et al*. 2009; Dadi *et al*. 2012).

CONCLUSION

SNPs on bovine genome were successfully identified across total chromosomes of cattle breeds of Ongole Grade (OG) and Holstein Friesian (HF). Some SNP markers with high MAF scores (> 0.2) revealed

approximately 69% and could be useful in genetic diversity analyses, given their great differentiating power. Several SNPs within and close to genes may provide an excellent solution to the disadvantage of SNP markers that have been used in diversity analyses. Dairy and beef cattle possessed specific alleles corresponding to known genes which may contribute to the genetic characters of each breed. The Bovine SNP 50K described in this study was more usable for differentiation among breeds than individuals in the same breed of cattle.

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