



# Comprehensive assessment of genetic diversity, structure, and relationship in four Japanese cattle breeds by Illumina 50K SNP array analysis

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1 **Original Article**

2 **Comprehensive assessment of genetic diversity, structure, and relationship in four**  
3 **Japanese cattle breeds by Illumina 50K SNP array analysis**

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## 1 **ABSTRACT**

2       There are four unique cattle breeds in Japan: Japanese Black, Japanese Brown,  
3 Japanese Polled, and Japanese Shorthorn. The objective of this study was to  
4 comprehensively assess the genetic diversity, structure, relationship, and the degree of  
5 influence from foreign breeds (Angus, Simmental, Hanwoo, Shorthorn, Ayrshire, Brown  
6 Swiss, and Devon) in the Japanese cattle breeds using Illumina 50K SNP array. In  
7 principal component analysis, each Japanese breed was separately clustered except for  
8 Japanese Shorthorn and Shorthorn. Japanese cattle breeds also showed different genetic  
9 components from each other at  $K \geq 5$  in population structure analysis. Japanese Shorthorn,  
10 on the other hand, had a very similar structure to Shorthorn at  $K \leq 9$ , and Japanese Polled  
11 had a partially similar component with Angus at  $K = 3-7$ . Such close relationships were  
12 also observed in the phylogenetic tree. These findings imply that Japanese cattle breeds  
13 share genetic components with European cattle breeds to some extent, while they have  
14 been almost differentiated. In population structure analysis, Japanese Black cattle shared  
15 little genetic component (3.5%) with European breeds. This is the first study to determine  
16 the extent to which European breeds impact Japanese breeds.

17

18 **Keywords:** *50K SNP array, genetic diversity, genetic relationship, genetic structure,*

1 *Japanese cattle breeds*

## 1 INTRODUCTION

2 In Japan, there are four unique cattle breeds: Japanese Black, Japanese Brown,  
3 Japanese Polled, and Japanese Shorthorn cattle. Due to crossbreeding with several foreign  
4 cattle breeds into Japanese indigenous cattle during the dawn of breeding improvement  
5 in the early 20th century, they have varied genetically (Gotoh *et al.*, 2014; Mannen *et al.*,  
6 2017; Sasazaki *et al.*, 2006). Some imported European cattle breeds, such as Simmental,  
7 Devon, Brown Swiss, and Ayrshire, were temporarily crossbred with Japanese Black  
8 cattle. However, because hybrid cattle require more feed than native cattle, the original  
9 importation strategy failed, and the Japanese government swiftly removed crossed cattle  
10 from herds to re-create totally pure breeds (Mukai *et al.*, 1989; Mannen *et al.*, 1998).  
11 Nevertheless, some genetic influence from the European breed may remain. Japanese  
12 Brown cattle are categorized into two strains: Kochi-strain and Kumamoto-strain, which  
13 are bred in Kochi Prefecture and Kumamoto Prefecture, respectively. These strains were  
14 independently established in each prefecture by crossbreeding with Simmental, Devon,  
15 and Hanwoo (Honda *et al.*, 2006). As a result, these two strains have different phenotypes  
16 in parts. For instance, the Kochi-strain has a brown body with some black parts such as  
17 eyelids, horns, and a nose, while the color of the Kumamoto-strain is solid brown. The  
18 Japanese Polled and Japanese Shorthorn were established by crossbreeding with

1 Aberdeen Angus and Shorthorn, respectively. These are bred in limited areas, Japanese  
2 Polled in Yamaguchi Prefecture (Mannen, 2017) and Japanese Shorthorn in Tohoku and  
3 Hokkaido regions (Noda *et al.*, 2018).

4 The genetic structure of Japanese cattle breeds has been studied based on the sequence  
5 of the mtDNA D-loop region in previous studies. They discovered that the unique  
6 haplogroup T4 was detected in all Japanese cattle breeds, with frequency exceeding half  
7 (Mannen *et al.*, 1998, 2004, 2017; Noda *et al.*, 2018; Sasazaki *et al.*, 2006). In addition,  
8 Noda *et al.* (2018) discovered that a rare haplogroup P was also detected in Japanese  
9 Shorthorn. These results in previous studies revealed that all Japanese cattle breeds may  
10 have distinct genetic components and structures from those of foreign cattle breeds.

11 Meanwhile, a few studies were carried out to investigate the genetic relationships  
12 between Japanese and foreign cattle breeds using autosomal single nucleotide  
13 polymorphisms (SNPs). Using genotyped data of 117 autosomal SNPs, Yonesaka *et al.*  
14 (2016) reported that Japanese Polled and Japanese Shorthorn were clustered with  
15 European cattle breeds, suggesting strong genetic influences from European breeds  
16 during the establishment of these two Japanese breeds. Furthermore, Sharma *et al.* (2016)  
17 reported that American Wagyu and Korean cattle were genetically close according to 50K  
18 SNP array data analysis. These two previous studies showed the close relationships

1 between Japanese and some foreign cattle breeds, though mitochondrial analysis implied  
2 the unique genetic components of Japanese breeds. This suggested that further  
3 investigation would be required using enormous SNPs data covering the whole genome,  
4 in addition to mtDNA analysis, to elucidate their genetic structures and relationships.

5 However, there is no study which investigated on all four Japanese cattle breeds at the  
6 same time using 50K SNP array analysis. Furthermore, no study has investigated the  
7 degree of genetic influences from foreign cattle breeds on each Japanese breed. Thus, our  
8 objective in the current study was to comprehensively assess the genetic diversity,  
9 structure, relationship, and the degree of influence from foreign breeds in all four  
10 Japanese cattle breeds using 50K SNP array data.

11

## 12 **MATERIALS AND METHODS**

### 13 **Ethics**

14 The experiments in the current study were carried out according to the Kobe University  
15 Animal Experimentation Regulations. All samples were collected by veterinarians or  
16 individual livestock owners. These treatments were carried out in accordance with  
17 Japanese Veterinarians Act (Act No. 186 of 1949).

18

## 1 **Collected samples and downloaded data**

2 We collected DNA samples from all Japanese cattle breeds; 48 samples from Japanese  
3 Black, 42 samples from Japanese Brown Kochi-strain, 48 samples from Japanese Brown  
4 Kumamoto-strain, 48 samples from Japanese Polled, and 48 samples from Japanese  
5 Shorthorn (Table S1). The samples of Japanese Black were collected at the 9<sup>th</sup> National  
6 Competitive Exhibition of Wagyu (Zenkoku Wagyu Noryoku Kyoshinkai), which was  
7 entered by Japanese Black cattle bred in all over Japan (a total of 34 prefectures). The  
8 samples of Japanese Brown Kochi-strain, Japanese Brown Kumamoto-strain, Japanese  
9 Polled, and Japanese Shorthorn were collected from animals bred in Kochi, Kumamoto,  
10 Yamaguchi, and Iwate Prefecture, respectively. Although animals of these Japanese  
11 breeds were somewhat related to each other within each breed, we selected as unrelated  
12 samples as we can by random selection. The samples of Japanese Shorthorn were also  
13 used in a previous study (Noda *et al.* 2018). DNA was extracted from blood, nasal  
14 secretion, or fat tissue. Meanwhile, we downloaded genotyping data of 20 Angus, 20  
15 Simmental, 8 Hanwoo, 17 Shorthorn, 18 Ayrshire, 12 Brown Swiss, and 4 Devon from  
16 dryad.org (<https://datadryad.org/stash/dataset/doi:10.5061/dryad.th092>). They were  
17 genotyped using BovineSNP50 BeadChip Ver.1 (Illumina, San Diego, CA, USA) in a  
18 previous study (Decker *et al.*, 2014).



1

## 2 **Genotyping and quality control**

3 All Japanese cattle breed samples were genotyped using each of three versions (*i.e.*  
4 Ver.1, Ver.2, and Ver.3) of BovineSNP50 BeadChip (Illumina, San Diego, CA, USA). The  
5 Ver.1 was applied for all samples of 48 Japanese Black and 10 Japanese Brown Kochi-  
6 strain, the Ver.2 for 48 Japanese Brown Kumamoto-strain and the Ver.3 for 32 Japanese  
7 Brown Kochi-strain, 48 Japanese Polled, and 48 Japanese Shorthorn. Common 40,329  
8 autosomal SNPs contained in all versions were selected based on positions on UMD3.0  
9 and SNP\_name. Moreover, some allele representations even for the same SNP varied  
10 depends on the versions because of different strands. We therefore rewrote alleles of such  
11 the SNPs as the consistent allele and excluded SNPs for which the strand of allele was  
12 unclear.

13 One individual of Japanese Brown Kochi-strain was excluded due to its call rate  $< 0.95$   
14 based on the genotyping results. All SNPs subsequently went through quality control  
15 using PLINK v.1.9 (Purcell *et al.*, 2007). The exclusion criteria for SNPs were call rate  $<$   
16  $0.95$ , minor allele frequencies (MAF)  $< 0.01$ , and Hardy-Weinberg equilibrium  $< 0.001$ .  
17 A total of 27,007 SNPs was finally used to calculate indices of genetic diversity, perform  
18 principal component analysis (PCA), and construct the phylogenetic tree in the current

1 study.

2

### 3 **Statistical analysis**

4 The average of MAF, expected ( $H_e$ ) and observed ( $H_o$ ) heterozygosities, and the  
5 inbreeding coefficient ( $F_{is}$ ) for each SNP were calculated in each breed using PLINK  
6 v.1.9., followed by the calculation of the average for all SNPs as indices of genetic  
7 diversity for each breed. PCA was performed in PLINK v.1.9. from all allele frequencies  
8 in each breed.

9 Population structure analysis was conducted in ADMIXTURE v1.3.0 to determine the  
10 population structure and degree of admixture among the twelve breeds (Alexander *et al.*,  
11 2009). For evaluating the model fitting, the cross-validation (CV) procedure was  
12 performed by 5-fold CV. Prior to the analysis, LD pruning was applied for the 27,007  
13 SNPs to exclude SNPs of  $r^2 > 0.5$  (Blachburn *et al.* 2017), resulting in 14,355 SNPs. We  
14 performed 11 independent runs for each K (from 2 to 12), followed by the visualizing in  
15 CLUMPAK server (<http://clumpak.tau.ac.il/>). We additionally applied the population  
16 structure analysis for eight cattle breeds including Japanese Black and seven foreign cattle  
17 breeds for the estimation of the genetic influence from foreign breeds for Japanese Black  
18 cattle in detail. In the additional analysis, the result at  $K = 3$  is the most informative to

1 estimate the genetic influence, and thus, the average of estimated ancestry proportions  
2 (%) for each cattle breed were calculated to observe the admixture proportions of foreign  
3 cattle breeds for Japanese Black at  $K = 3$ .

4 To investigate the phylogenetic relationships among populations, the standard genetic  
5 distance of Nei (Nei, 1972) were calculated in RStudio v4.0.2 to construct the  
6 phylogenetic tree using the unweighted pair group method with arithmetic mean  
7 (UPGMA) (Sneath & Sokal, 1973). In the phylogenetic relationship analysis, we included  
8 *Bos indicus* ( $n = 10$ ), which was genotyped using BovineSNP50 BeadChip, as an  
9 outgroup.

10

## 11 **RESULTS**

12 In all breeds, we determined allele frequencies (Table 1). In Japanese cattle breeds, the  
13 mean MAF ranged from 0.196 to 0.213 and the mean  $H_e$  and  $H_o$  ranged from 0.262 to  
14 0.284 and from 0.283 to 0.299, respectively. In foreign cattle breeds, the mean MAF  
15 ranged from 0.197 to 0.234 and the mean  $H_e$  and  $H_o$  ranged from 0.267 to 0.311 and from  
16 0.269 to 0.322, respectively. The  $F_{is}$  ranged from -0.137 to -0.006 among all breeds.

17 According to PCA, animals of the same breed were nearby plotted and each breed  
18 nearly made an independent cluster except for Japanese Shorthorn and Shorthorn (Figure

1 1, S1). Whereas Asian cattle breeds and European cattle breeds were almost distinguished  
2 by PC1 (5.18%), Japanese Polled and Japanese Shorthorn were plotted close to European  
3 breeds in the PC1. PC2 (4.27%) was the single factor that distinguished Japanese Polled  
4 from other breeds. PC3 (3.34%) significantly distinguished Japanese Black, Japanese  
5 Brown Kochi-strain, and Japanese Brown Kumamoto-strain. Japanese Shorthorn was  
6 clustered with Shorthorn by any PCs.

7 The results of population structure analysis were showed in Figure 2. The CV error  
8 plot (Figure S2) indicates the higher K values exhibit the lower CV errors at K=1-7, and  
9 reaches the plateau at  $K > 7$ . Japanese Black, Japanese Brown Kochi-strain, and Japanese  
10 Brown Kumamoto-strain were clustered at  $K = 2$ , and Japanese Polled and European  
11 breeds showed similar and admixed patterns. At practically all K's, Japanese Shorthorn  
12 was clustered with Shorthorn. Japanese Polled had a nearly solid pattern at  $K = 3$ , and its  
13 component was admixed in Japanese Black, Hanwoo, and European breeds. Two strains  
14 of Japanese Brown were identified at  $K = 4$ . At  $K = 5$ , Japanese Black made an  
15 independent cluster but only Hanwoo has this component. Japanese breeds showed almost  
16 independent component at  $K \geq 5$ , whereas European breeds showed admixed patterns.  
17 The genetic component dominant in European breeds were observed at  $K \geq 6$ . From  $K =$   
18 7 to 9, European breeds were gradually separated. Japanese Shorthorn and Shorthorn were

1 separated at  $K \geq 10$ .

2 An additional population structure analysis was conducted to estimate the degree of  
3 genetic influence from foreign cattle breeds on Japanese Black cattle. The result at  $K = 3$   
4 would seem to be appropriate to estimate it. According to the result, the mean admixed  
5 proportion in Japanese Black cattle was 3.5% (Figure 3, Table S2).

6 We illustrated the phylogenetic tree using UPGMA method based on Nei's genetic  
7 distance (Figure 4). As the result, Japanese Black, Japanese Brown Kochi-strain, Japanese  
8 Brown Kumamoto-strain, and Hanwoo were clustered as Asian cattle breeds. Among  
9 these Asian breeds, Japanese Black and Hanwoo were nearby located in the cluster.  
10 Meanwhile, Japanese Polled was clustered with Angus. Furthermore, similar with the  
11 results of the other analyses, Japanese Shorthorn was clustered with Shorthorn.

12

## 13 **DISCUSSION**

14 In the current study, we investigated on genetic diversities, structures, and relationships  
15 in Japanese cattle breeds using 50K SNP array data. Although they have been reported in  
16 prior research, based on sequences of mtDNA D-loop region, SNPs on Y chromosome,  
17 and several hundred autosomal SNPs (Mannen *et al.*, 1998, 2004, 2006, 2017; Noda *et*  
18 *al.*, 2018; Sasazaki *et al.*, 2006; Yonesaka *et al.* 2016), this is the first study to

1 comprehensively investigate using 50K SNP array data in all four Japanese cattle breeds.  
2 We also used 50K SNP datasets of foreign cattle breeds downloaded from a database to  
3 compare the genetic diversity and survey the genetic relationship between Japanese and  
4 foreign breeds. Although the number of datasets for the foreign breeds was relatively  
5 small, we were able to assess the broad relationships as with previous studies (Alam *et*  
6 *al.*, 2021; Decker *et al.*, 2014; Strucken *et al.*, 2021). However, the number of datasets of  
7 Devon was significantly small, and therefore, we payed attention considering the small  
8 number in assessment of the relationships between Devon and the other breeds in this  
9 study.

10 The indicators of genetic diversity (the mean MAF,  $H_e$ , and  $H_o$ ) in Japanese cattle  
11 breeds (MAF: 0.196–0.213,  $H_e$ : 0.262–0.284,  $H_o$ : 0.283–0.299) were relatively lower  
12 than those in the foreign breeds (MAF: 0.197–0.234,  $H_e$ : 0.259–0.311,  $H_o$ : 0.269–0.322).  
13 This was consistent with some previous studies showing that Japanese cattle breeds had  
14 low genetic diversity than European and Korean cattle breeds based on sequences of  
15 mtDNA D-loop region and autosomal SNPs (Mannen *et al.*, 2004; Sharma *et al.*, 2016;  
16 Yonesaka *et al.*, 2016). In Japanese Black, the inbreeding coefficient has recently  
17 increased mainly due to the intensive use of a few sires for the improvement of beef  
18 quantity and quality, and therefore its genetic diversity has decreased (Honda *et al.*, 2001).

1 Meanwhile, the genetic diversity of the Japanese Brown Kochi-strain, Japanese Brown  
2 Kumamoto-strain, Japanese Shorthorn, and Japanese Polled was minimal, owing to the  
3 limited size of the populations (Nagamine *et al.*, 1997; Honda *et al.*, 2006; Mannen, 2017).  
4 For example, the Japanese Brown Kochi-strain has been maintained on less than 500  
5 individuals (Honda *et al.*, 2006). Since the genetic diversity in the Japanese breeds is  
6 going to decrease, we would be required to constantly observe the genetic diversity to  
7 avoid the decrease.

8 In PCA, almost all breeds were separately clustered. Although Japanese Black and  
9 Japanese Brown (Kochi-strain and Kumamoto-strain) were nearly plotted in PC1 and PC2,  
10 they were completely separated in PC3. We assumed that these Japanese cattle breeds  
11 would not have such strong relationships because PC1, PC2, and PC3 explained similar  
12 proportions (5.18%, 4.27%, and 3.34%, respectively) and we observed similar results in  
13 PC4 (3.00%, data not shown) and PC5 (2.54%, data not shown) with the result in PC3.  
14 Furthermore, the Japanese cattle breed clusters were wider apart than the foreign cattle  
15 breed clusters. In addition to the PCA, population structure analysis revealed that the  
16 Japanese breeds have the different genetic structures from each other at  $K \geq 5$ , while the  
17 foreign breeds showed the similar genetic structures. These results suggested that all  
18 Japanese cattle breeds have distinct genetic characteristics.

1 We detected a unique genetic structure in Japanese Polled by PCA and population  
2 structure analyses. This breed has been maintained as small population ( $< 200$ ) in a  
3 limited area of Yamaguchi Prefecture, Japan (Mannen *et al.*, 2017). According to a  
4 previous study, the genetic drift happens faster in small population than larger one (Masel,  
5 2011). The small population size might cause the great genetic drift in Japanese Polled,  
6 and therefore the breed has been differentiated from the other cattle breeds. However, the  
7 population structure analysis at  $K = 6$  and  $7$  found a dominating genetic component in  
8 Japanese Polled in Angus, and these two breeds were clustered in the phylogenetic tree  
9 (Figure 4). Japanese Polled was established by crossbreeding with imported Angus to  
10 improve its carcass traits (Mannen, 2017). Based on the breeding history and the results  
11 in the current study, this breed shares some genetic components with Angus, while it has  
12 been differentiated by genetic drift.

13 Japanese Shorthorn showed the quite similar genetic structure with Shorthorn in PCA,  
14 population structure analysis, and the phylogenetic tree, while Japanese Shorthorn had  
15 distinct mitochondrial haplogroups P (Noda *et al.*, 2018). This difference was due to  
16 genetic markers used in each study, *i.e.* autosomal markers in the current study and  
17 mitochondrial markers in the previous study. Whereas mitochondrial markers would  
18 useful for estimation of maternal origin, autosomal markers are important to investigate



1 genetic structure and relationships within and among breeds. Japanese Shorthorn was  
2 established by crossbreeding of indigenous cattle “Nambu” bred in Tohoku region in  
3 Japan with Shorthorn imported from America (Noda *et al.*, 2018). The results in the  
4 current study suggested that this breed would have the strong genetic influence of  
5 Shorthorn. Furthermore, in terms of origin, the ancestor of the Japanese Shorthorn  
6 “Nambu” is thought to be distinct from the common ancestors of the other Japanese cattle  
7 breeds (Yamada, 1922; Noda *et al.*, 2018). This historical context could also explain the  
8 apparent genetic differences between the various Japanese cattle breeds.

9 In population structure analysis, the two strains of Japanese Brown and Japanese Black  
10 cattle showed almost solid patterns at  $K \geq 5$ , indicating that these breeds are not  
11 influenced by foreign cattle breeds. With these Japanese breeds, only Hanwoo showed  
12 the same genetic components. In particular, Japanese Black and Hanwoo were observed  
13 in almost the same cluster in the PCA and phylogenetic tree. This result was consistent  
14 with some previous studies (Sharma *et al.*, 2016; Lin *et al.*, 2010; Lee *et al.*, 2014),  
15 implying that despite no crossbreeding, these two cattle breeds would have similar genetic  
16 constructions. Furthermore, the result in population structure analysis showed that they  
17 shared about 50% of their genetic constructions (Figure 3, Table S2). Based on these  
18 results, Japanese Black and Hanwoo may share a genetic component acquired from a

1 common ancestor during the migration period across the Korean Peninsula to Japan.  
2 Meanwhile, population structure analysis revealed that Japanese Black shared 3.5% of  
3 their genetic constructions with European cattle breeds. Though the value might not be  
4 so accurate, the result suggested that the influence of crossbreeding is little for Japanese  
5 Black cattle. The recent breeding system, which is fully isolated to other cattle breeds,  
6 has reduced genetic impacts from European cattle breeds, resulting in a small genetic  
7 component.

8 As for sub-structures within Japanese Black, Japanese Brown Kochi-strain and  
9 Japanese polled, they show the disappearance and reappearance at  $K \geq 9$ . The CV error  
10 plot (Figure S2) suggests the model fitting improved in ascending order of  $K$  (up to 7),  
11 and fittings reach plateau at  $K > 7$ . Therefore, the instable appearance/ disappearance of  
12 sub-structures within Japanese breeds are not due to model misspecification caused by  
13 the parameter-overfitting. It is noteworthy that the sub-structures within Japanese breeds  
14 are fundamentally descended from unique sources, and the reappeared sub-structures are  
15 essentially consistent with the ones before disappearances. These observations suggest  
16 the hierarchical nested relationships in the population structure are stable. Accordingly,  
17 the instability may not be due to the instable inferences of genetic structure itself, but the  
18 instable exposure of the “hidden” genetic structure consists of the multiple sub-structures

1 with similar levels of genetic differentiation ( $F_{st} = 0.079\sim 0.109$ ). Japanese cattle breeds  
2 have been isolated from other breeds and maintained by a limited number of sires since  
3 the breed establishment, leading to the lower genetic diversity. Such the genetic  
4 background would cause the sub-structures in each breed.

5 The genetic relationships illustrated in the current study were almost consistent with  
6 the results based on 117 autosomal SNPs (Yonesaka *et al.*, 2016). Accordingly, we might  
7 not need so abundant SNPs data to broadly assess the relationships. On the other hand,  
8 we should use much genetic information to estimate the accurate degree of influences and  
9 admixtures from other breeds. Further investigation using whole-genome sequences  
10 might provide more accurate evaluation.

11 The results in the current study revealed that all the Japanese cattle breeds have distinct  
12 genetic structure from each other, implying that the Japanese cattle breeds have been  
13 almost differentiated from indigenous cattle in Japan. On the other hand, they shared a  
14 part of genetic component with the foreign cattle breeds which were established from the  
15 same ancestor or crossbred with the Japanese cattle breeds. In addition, for the first time,  
16 the degree of influence from foreign breeds was measured, particularly in Japanese Black  
17 cattle. As the result, Japanese Black showed little influence, whereas Japanese Shorthorn  
18 was strongly influenced by Shorthorn. Further research into the genetic structure of each

- 1 breed would provide further knowledge useful for improving breeding and maintaining
- 2 genetic diversity.

## 1 **ACKNOWLEDGMENTS**

2 We thank Wagyu Registry Association for their sampling and their data collection.

3

## 4 **CONFLICT OF INTEREST**

5 There is no conflict of interest relevant to the contents in the current study.

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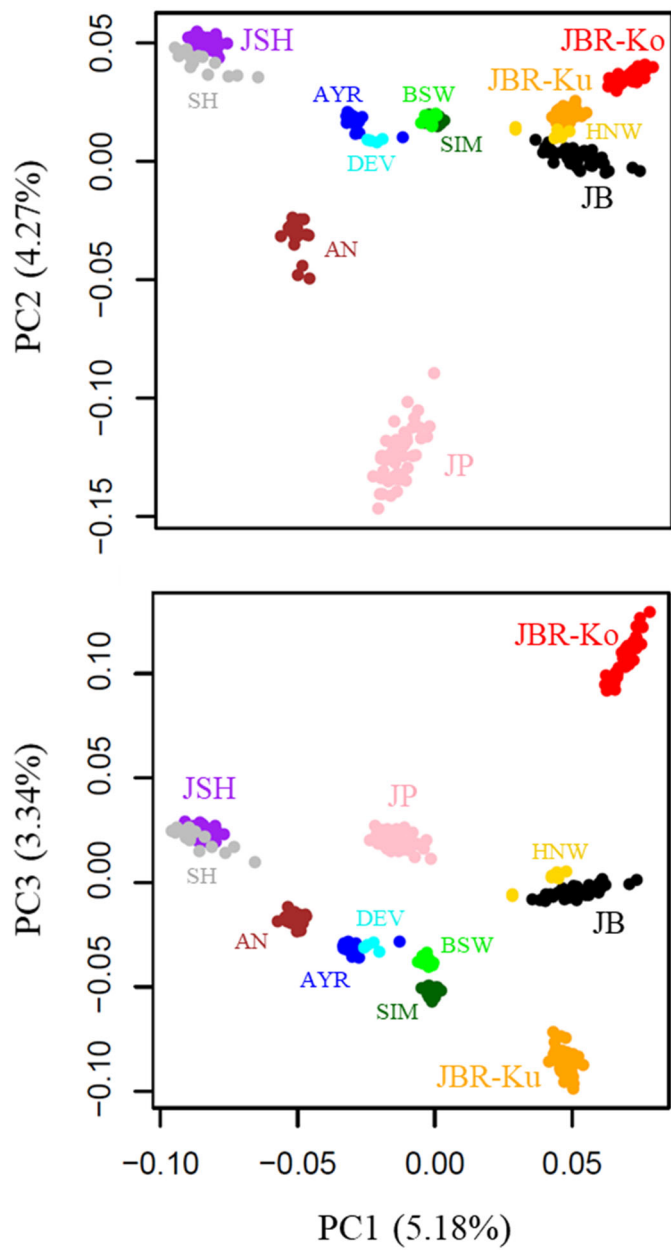
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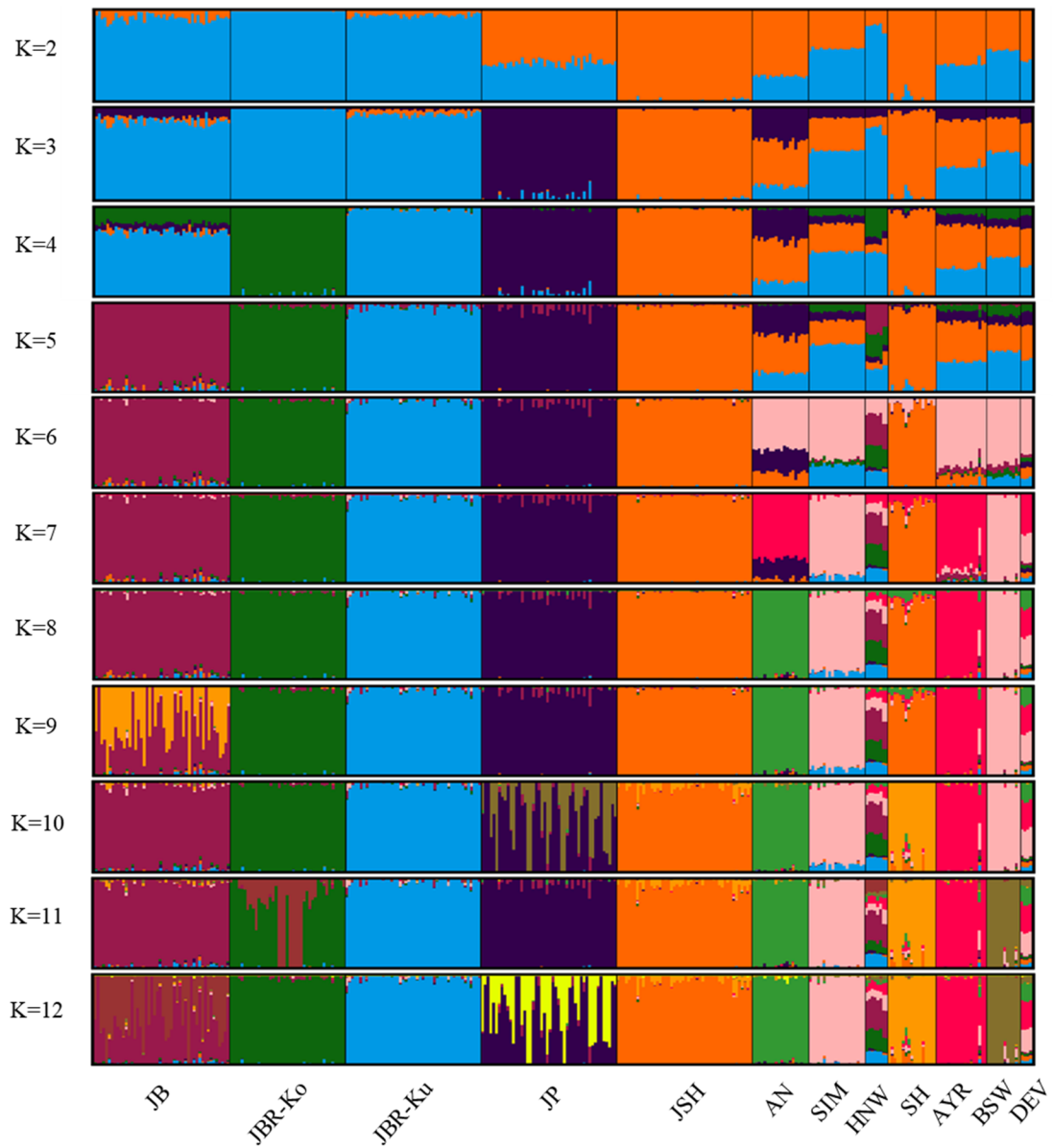
**Table 1** Indices of genetic diversity within each cattle breed

Breed	n	MAF	$H_e$	$H_o$	$F_{is}$
JB	48	0.207	0.278	0.287	-0.034
JBR-Ko	42	0.196	0.262	0.283	-0.080
JBR-Ku	48	0.213	0.284	0.299	-0.055
JP	48	0.203	0.269	0.291	-0.082
JSH	48	0.209	0.280	0.290	-0.035
HNW	8	0.212	0.282	0.307	-0.088
AN	20	0.234	0.311	0.322	-0.033
SIM	20	0.228	0.305	0.319	-0.049
SH	17	0.198	0.267	0.269	-0.006
AYR	18	0.230	0.306	0.321	-0.049
BSW	12	0.208	0.278	0.303	-0.092
DEV	4	0.197	0.259	0.294	-0.137

MAF: mean minor allele frequencies,  $H_o$ : observed heterozygosity,  $H_e$ : expected heterozygosity,  $F_{is}$ : inbreeding coefficient, JB: Japanese Black, JBR-Ko: Japanese Brown Kochi-strain, JBR-Ku: Japanese Brown Kumamoto-strain, JP: Japanese Polled, JSH: Japanese Shorthorn, HNW: Hanwoo, AN: Angus, SIM: Simmental, SH: Shorthorn, AYR: Ayrshire, BSW: Brown Swiss, DEV: Devon.

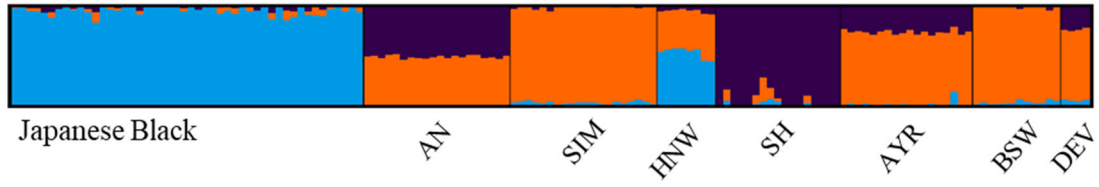


**Figure 1** Principal component analysis (PCA) in Japanese and foreign cattle breeds  
 JB: Japanese Black, JBR-Ko: Japanese Brown Kochi-strain, JBR-Ku: Japanese Brown Kumamoto-strain, JP: Japanese Polled, JSH: Japanese Shorthorn, HNW: Hanwoo, AN: Angus, SIM: Simmental, SH: Shorthorn, AYR: Ayrshire, BSW: Brown Swiss, DEV: Devon.

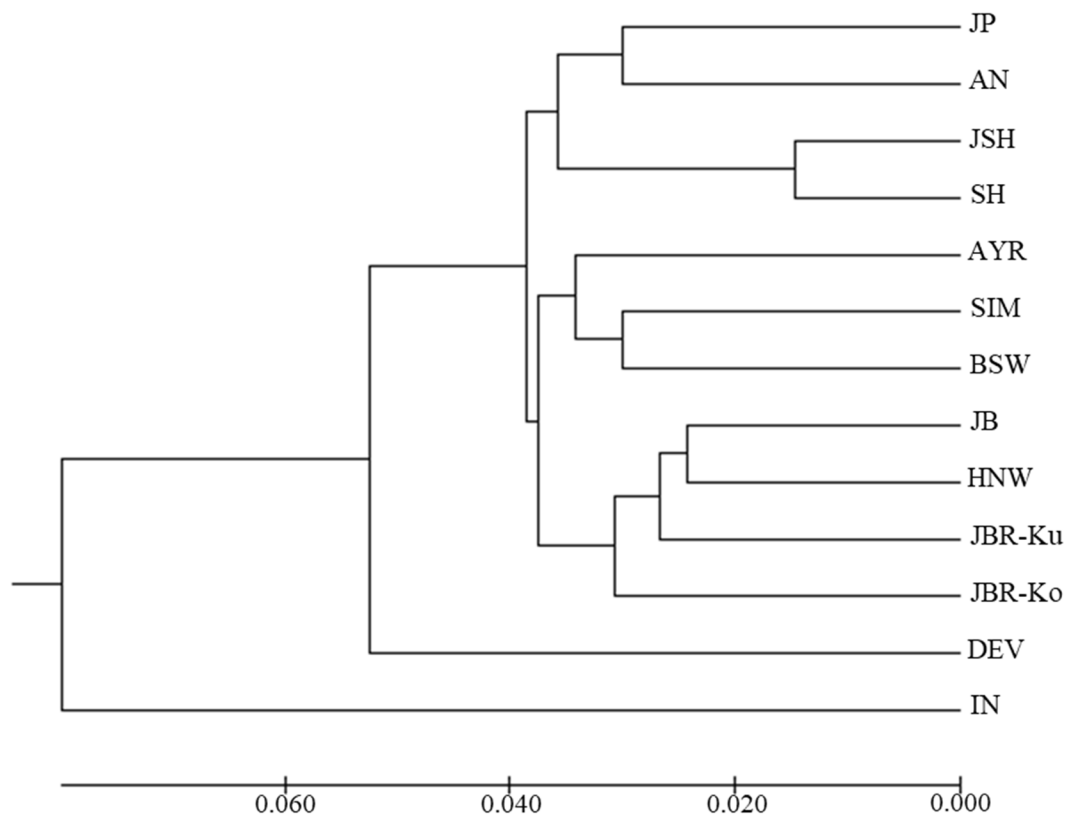


**Figure 2** Genetic structure of Japanese and foreign cattle breeds at  $K = 2 - 12$

JB: Japanese Black, JBR-Ko: Japanese Brown Kochi-strain, JBR-Ku: Japanese Brown Kumamoto-strain, JP: Japanese Polled, JSH: Japanese Shorthorn, HNW: Hanwoo, AN: Angus, SIM: Simmental, SH: Shorthorn, AYR: Ayrshire, BSW: Brown Swiss, DEV: Devon.



**Figure 3** Genetic structure of Japanese Black and 7 foreign cattle breeds at  $K = 3$   
 AN: Angus, SIM: Simmental, HNW: Hanwoo, SH: Shorthorn, AYR: Ayrshire, BSW: Brown Swiss,  
 DEV: Devon.



**Figure 4** Phylogenetic tree using UPGMA method based on Nei's genetic distance  
 JB: Japanese Black, JBR-Ko: Japanese Brown Kochi-strain, JBR-Ku: Japanese Brown Kumamoto-strain, JP: Japanese Polled, JSH: Japanese Shorthorn, HNW: Hanwoo, AN: Angus, SIM: Simmental, SH: Shorthorn, AYR: Ayrshire, BSW: Brown Swiss, DEV: Devon, IN: *Bos indicus* (out group).