Cattle were introduced from North China to the Korean peninsula and Japan around A.D. 200 (Kim and Lee, 2000). Three cattle heads with brown, brindle or black coat colors, are estimated to be the origin of the Korean cattle breeds (Na, 2008). At present, four cattle breeds, Hanwoo, Chikso, Heugu, and Jeju black, exist in Korea, and are classified based on their different coat colors and geographical distribution (Suh et al., 2014). These breeds have been documented in the Domestic Animal Diversity Information System (DAD-IS) of the United Nations Food and Agriculture Organization (FAO; http://dad.fao.org/).

Because the three Korean native cattle breeds, except for Hanwoo, were recently recognized as possessing valuable genetic resources in Korea, molecular studies were performed on them using genetic markers for characteristic evaluation.

Various molecular genetic markers have been used to determine genetic diversity, molecular evolution and genetic relationship within and among cattle breeds (Horsburgh et al., 2013; Ludwig et al., 2013; San et al., 2014; Tu et al., 2014; Xin et al., 2014; Hristov et al., 2015). Of these, mitochondrial DNA (mtDNA) contains a maternal genetic pattern, and is a very useful marker for evaluating the origin and gene flow of modern cattle breeds. Especially,

**ABSTRACT:** Many studies have reported the frequency and distribution of haplogroups among various cattle breeds for verification of their origins and genetic diversity. In this study, 318 complete sequences of the mtDNA control region from four Korean cattle breeds were used for haplogroup classification. 71 polymorphic sites and 66 haplotypes were found in these sequences. Consistent with the genetic patterns in previous reports, four haplogroups (T1, T2, T3, and T4) were identified in Korean cattle breeds. In addition, T1a, T3a, and T3b sub-haplogroups were classified. In the phylogenetic tree, each haplogroup formed an independent cluster. The frequencies of T3, T4, T1 (containing T1a), and T2 were 66%, 16%, 10%, and 8%, respectively. Especially, the T1 haplogroup contained only one haplotype and a sample. All four haplogroups were found in Chikso, Jeju black and Hanwoo. However, only the T3 and T4 haplogroups appeared in Heugu, and most Chikso populations showed a partial of four haplogroups. These results will be useful for stable conservation and efficient management of Korean cattle breeds. (**Key Words:** Haplogroup, mtDNA, Control Region, Korean Cattle Breed, Phylogenetic Tree)
this marker has been used for the haplogroup classification of modern and extinct cattle (Gou et al., 2010; Dadi et al., 2012; Gravlund et al., 2012). At present, 6 haplogroups (T, T1, T2, T3, T4, and T5) have been reported among Europe, Africa, and Near East, and Asia cattle populations (Troy et al., 2001; Mannen et al., 2004; Achilli et al., 2008). The T3 haplogroup is distributed mainly in Europe and Asia, whereas T1 is found in Africa, T and T2 in the Near East, and T4 in the Northeast.

Korean cattle have been classified into the T2, T3, and T4 haplogroups, but mainly into the T3 haplogroup (Lai et al., 2006; Sasazaki et al., 2006; Jia et al., 2010). However, these studies did not classify the haplogroup at the breed level for Korean cattle. Therefore, the aims of the present study were to analyze the mtDNA control region sequence and to classify the mtDNA haplogroup of each Korean cattle breed.

MATERIALS AND METHODS

Sample collection and DNA extraction

A total of 288 animals (182 Chikso, 20 Heugu, and 86 Jeju black) were used in this study. Chikso samples were collected from 6 Management Institutes, Gyeonggi Livestock and Veterinary Service (GG), Gangwon Provincial Livestock Research Institute (GW), Chungbuk Institute of Livestock and Veterinary Research (CB), Jeonbuk Institute of Livestock and Veterinary Research (JB), Chungnam Institute of Livestock Experiment Research (CN), and Jeonnam Agricultural Research and Extension Services (JN). Heugu and Jeju black samples were collected from CB and the Jeju Special Self-Governing Province Livestock Promotion (JJ), respectively. All samples were collected from each institute for detailed identification of haplogroups among the populations. The sequences for the Hanwoo breed were obtained from the GenBank database (http://www.ncbi.nlm.nih.gov/), distributed in 6 regions throughout Korea (Jeju, Jungeup, Cheongwon, Incheon, Youngju, and Yeoju) (AY337521-26, AY337529-41, AY337543-46). Genomic DNA was extracted from blood using the DNeasy Blood Kit (Qiagen, Hilden, Germany). The concentration of genomic DNA was measured using a NanoDrop ND 100 spectrometer (Thermo Scientific, Waltham, MA, USA).

DNA amplification and sequencing

Two primer sets were designed using the sequences for tRNA-Pro, the control region, and tRNA-Phe, according to the mtDNA genome sequences (GenBank accession no. V00654). The sequences of the two primer sets were as follows:

Set 1: 5'- ACCCCCCAAGCCTGAAGTCTTCT -3' and 5'-AGATGAGATGCCCTGAAGA-3';

Set 2: 5'- GGGTCGCTATCCAATGAATT -3' and 5'-GCATTTCAGTGCCTGTCTT -3'.

Polymerase chain reaction (PCR) was carried out using 2.5 μl of 10x reaction buffer, 0.2 mM of dNTP, 1.5 mM MgCl2, 1.5 units of Taq DNA polymerase (Takara, Tokyo, Japan), 10 mM of each primer, and 10 ng of genomic DNA in a final volume of 25 μl. The PCR amplification was performed using a PTC-200 (MJ Research, Waltham, MA, USA) under the following conditions: 35 cycles of 30 s at 94°C, 30 s at 63°C, and 60 s at 72°C. After purification of the PCR products using the QIAEX II Gel Extraction Kit (Qiagen, Germany), nucleotide sequencing was carried out by direct-sequencing with an ABI 3130xl Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA). The complete sequences of the complete control region were determined based on the complete sequences of the Bos taurus mtDNA genome sequence (Genbank accession no. V00654) as reported by Anderson et al. (1982). The determined sequences were deposited in the GenBank database (GenBank accession no. KR857524-74).

Data analysis

The sequences of the control region from four Korean cattle breeds were aligned in the CLUSTAL W program (Thompson et al., 1994) in the BioEdit software (Hall, 1999). Sites representing gaps in any of the aligned sequences were excluded from subsequent analyses. Identification of haplotypes was carried out using DNA sequence polymorphism version 5.1 (Librado and Rozas, 2009). Haplogroup classification was performed based on several previous reports (Troy et al., 2001; Achilli et al., 2008; Mannen et al., 2004). Genetic distances between haplotypes were estimated using the substitution model of Tamura and Nei (1993). The neighbor-joining (NJ) tree (Saitou and Nei, 1987) among haplotype sequences was reconstructed using the MEGA 5.05 package (Tamura et al., 2011).

RESULTS AND DISCUSSION

In this study, 288 complete sequences of the mtDNA control region were determined. In additions, 30 Hanwoo sequences were obtained from the GenBank database. The entire length of the control region ranged from 908 to 912 bp, because two insertion/deletion mutation sites were found at nucleotide positions 216 to 221 and 352 to 363 in the complete control region sequences (GenBank accession no. V00654). These regions contained two poly-C tracts, and the mutations were excluded from subsequent analyses. 71 polymorphic sites were detected and 66 haplotypes were classified (Figure 1). Of these, 7 (H1, H4, H6-8, H10, H39) were major haplotypes that contained more than 10 sequences (Table 1). In addition, breed- and population-specific sequences were found.
### Figure 1.
Sequence variations of 66 haplotypes in the mtDNA control region of four Korean cattle breeds. Mutations are scored relative to RS (GenBank accession no. V00654) of Anderson et al. (1982). Dots identify the RS sequence.

### Table 1.
i) Distribution of 66 haplotypes in four Korean cattle breeds based on sequence variations of the mtDNA control region

| Haplotypes | Breeds | Chikso | Heugu | Jeju Black | Hanwoo | Total | Accession no. |
|------------|--------|--------|-------|------------|--------|-------|---------------|
| H1         | GW     | 23     | 1     |            |        | 35    | KR857525      |
| H2         | CB     | 8      |       |            |        | 8     | KR857543      |
| H3         | CB     | 4      | 4     |            |        | 8     | KR857529      |
| H4         | CB     | 11     | 11    |            |        | 22    | KR857544      |
| H5         | CB     | 3      | 3     |            |        | 6     | KR857535      |
| H6         | CB     | 2      | 9     | 7          | 2      | 18    | KR857553      |
| H7         | CB     | 9      | 9     | 7          | 2      | 18    | KR857574      |
| H8         | CB     | 4      | 3     | 7          | 4      | 14    | KR857524      |
| H9         | CB     | 3      | 3     | 4          |        | 7     | KR857552      |
| H10        | CB     | 6      | 1     | 7          | 3      | 1     | KR857547      |
| H11        | CB     | 3      | 3     |            |        | 6     | KR857530      |
| H12        | CB     | 2      | 2     |            |        | 4     | KR857555      |
| H13        | CB     | 3      | 3     |            |        | 6     | KR857540      |
| H14        | CB     | 1      | 3     | 1          | 5      | 9     | KR857532      |
| H15        | CB     | 7      | 7     |            |        | 14    | KR857527      |
Table 1. ii) Distribution of 66 haplotypes in four Korean cattle breeds based on sequence variations of the mtDNA control region (Continued)

| Haplotypes | Breeds | Chikso | CB | GG | CN | JN | Sub-total | Heugu | Jeju Black | Hanwoo | Total Accession no. |
|------------|--------|--------|----|----|----|----|-----------|-------|------------|--------|---------------------|
| H16        |        | 2      | 2  |    |    |    |           |       |            |        | KR857534            |
| H17        |        | 7      | 1  | 8  |    |    |           |       |            |        | KR857570            |
| H18        |        | 3      | 3  |    |    |    |           |       |            |        | KR857541            |
| H19        |        | 4      | 1  | 5  |    |    |           |       |            |        | KR857536            |
| H20        |        | 1      | 1  |    |    |    |           |       |            |        | KR857565            |
| H21        |        | 7      | 1  | 8  |    |    |           |       |            |        | KR857564            |
| H22        |        | 3      |    | 3  | 5  | 1  |           |       |            |        | KR857572            |
| H23        |        | 1      |    | 1  |    |    |           |       |            |        | KR857526            |
| H24        |        | 1      |    | 1  |    |    |           |       |            |        | KR857537            |
| H25        |        | 1      |    | 1  |    |    |           |       |            |        | KR857557            |
| H26        |        | 1      |    | 1  |    |    |           |       |            |        | KR857561            |
| H27        |        | 2      | 2  | 4  |    |    |           |       |            |        | KR857531            |
| H28        |        | 1      |    | 1  |    |    |           |       |            |        | KR857538            |
| H29        |        | 1      |    | 1  |    |    |           |       |            |        | KR857568            |
| H30        |        | 1      |    | 1  |    |    |           |       |            |        | KR857546            |
| H31        |        | 5      |    | 5  |    |    |           |       |            |        | KR857528            |
| H32        |        | 1      | 4  | 5  | 3  | 8  |           |       |            |        | KR857563            |
| H33        |        | 2      | 2  |    |    |    |           |       |            |        | KR857542            |
| H34        |        | 1      | 1  |    |    | 2  |           |       |            |        | KR857550            |
| H35        |        | 1      |    | 1  |    |    |           |       |            |        | KR857533            |
| H36        |        |        | 2  | 2  |    |    |           |       |            |        | KR857539            |
| H37        |        |        | 4  |    |    | 4  |           |       |            |        | KR857554            |
| H38        |        | 8      |    |    |    | 8  |           |       |            |        | KR857545            |
| H39        |        | 18     | 1  | 19 |    |    |           |       |            |        | KR857573            |
| H40        |        | 9      |    |    |    | 9  |           |       |            |        | KR857569            |
| H41        |        | 3      |    | 3  |    |    |           |       |            |        | KR857560            |
| H42        |        | 1      |    | 1  |    |    |           |       |            |        | KR857548            |
| H43        |        | 1      |    | 1  |    |    |           |       |            |        | KR857562            |
| H44        |        | 1      |    | 1  |    |    |           |       |            |        | KR857567            |
| H45        |        | 1      |    | 1  |    |    |           |       |            |        | KR857556            |
| H46        |        | 1      |    | 1  |    |    |           |       |            |        | KR857551            |
| H47        |        | 1      |    | 1  |    |    |           |       |            |        | KR857559            |
| H48        |        | 1      |    | 1  |    |    |           |       |            |        | KR857558            |
| H49        |        | 1      |    | 1  |    |    |           |       |            |        | KR857549            |
| H50        |        | 1      |    | 1  |    |    |           |       |            |        | KR857566            |
| H51        |        | 1      |    | 1  |    |    |           |       |            |        | KR857571            |
| H52        |        | 1      |    | 1  |    |    |           |       |            |        | AY337546            |
| H53        |        | 1      |    | 1  |    |    |           |       |            |        | AY337545            |
| H54        |        | 1      |    | 1  |    |    |           |       |            |        | AY337543            |
| H55        |        | 1      |    | 1  |    |    |           |       |            |        | AY337540            |
| H56        |        | 1      |    | 1  |    |    |           |       |            |        | AY337539            |
| H57        |        | 1      |    | 1  |    |    |           |       |            |        | AY337537            |
| H58        |        | 4      |    |    |    | 4  |           |       |            |        | AY337535            |
| H59        |        | 1      |    | 1  |    |    |           |       |            |        | AY337532            |
| H60        |        | 1      |    | 1  |    |    |           |       |            |        | AY337530            |
| H61        |        | 1      |    | 1  |    |    |           |       |            |        | AY337526            |
| H62        |        | 1      |    | 1  |    |    |           |       |            |        | AY337525            |
| H63        |        | 1      |    | 1  |    |    |           |       |            |        | AY337524            |
| H64        |        | 1      |    | 1  |    |    |           |       |            |        | AY337523            |
| H65        |        | 1      |    | 1  |    |    |           |       |            |        | AY337522            |
| H66        |        | 1      |    | 1  |    |    |           |       |            |        | AY337521            |
| Total      | 51     | 51     | 12 | 7  | 18 | 182 | 20        | 86    | 30         | 318    |                     |

GW, Gangwon Provincial Livestock Research Center; CB, Chungbuk Institute of Livestock and Veterinary Research; JB, Jeonbuk Institute of Livestock and Veterinary Research; GG, Gyeonggi Livestock and Veterinary Service; CN, Chungnam Institute of Livestock and Veterinary Research; JN, Jeonnam Agricultural Research and Extension Services.
Consistent with the genetic pattern reported by Mannen et al. (2004) and Jia et al. (2010), four haplogroups (T1, T2, T3, and T4) were identified in Korean cattle breeds (Figure 2). T and T5 haplogroups have been defined by a transition at positions 16255 and 163 in reference sequences (RS, V00654), respectively (Troy et al., 2001; Achilli et al., 2008; Jia et al., 2010). However, these haplogroups were absent in this study. The T4 haplogroup was differentiated from T3 by a transition at position 16042, 16093, and 16302. Moreover, the T2 haplogroup was differentiated from T by a transition at position 16057 and a transversion at position 16185. T1, the African cattle specific haplogroup, was identified by a transition at position 16050 and 16113. In addition, T1a, a sub-haplogroup reported by Jia et al. (2010) and Achilli et al. (2008), differed in only one position, 16050.

The phylogenetic tree was constructed using 66 haplotype sequences (Figure 3). This phylogenetic pattern was compared with the classified haplogroups. Each haplogroup formed an independent cluster. The T3 haplogroup was classified into T3a and T3b sub-haplogroups by previous reports (Achilli et al., 2008; Jia et al., 2010). In this study, T3a and T3b were differentiated by a mutation pattern at position 169. Partial haplotypes contained in the T3b sub-haplogroups showed distinct mutation patterns in three positions (16122, 16055, and 16119). The T4 haplogroup was separate from the 3a sub-haplogroup, and this phylogenetical pattern agreed with previous reports (Achilli et al., 2008; Jia et al., 2010). The T1 haplogroup and the T1a sub-haplogroup were located together in an independent group.

Several studies have reported the frequency and

![Figure 3](image-url)
distribution of haplogroups among various cattle breeds in East Asia (Mannen et al., 2004; Lai et al., 2006; Sasazaki et al., 2006; Jia et al., 2010). T3 was confirmed as the major haplogroup in Chinese cattle. On the other hand, the T4 haplogroup predominates (about 65%) in Japanese cattle with approximately two fold higher frequency than T3. In Korean cattle, the frequency of the T3 haplogroup ranged from 69% to 83% and was the highest among the haplogroups. In this study, the haplotype distribution was confirmed among four Korean native cattle breeds (Table 2).

The frequencies of T3, T4, T1 (containing T1a), and T2 were 66%, 16%, 10%, and 8%, respectively. The frequency of the T3 haplogroup was 66%, which was lower than that of several previous reports (Mannen et al., 2004; Lai et al., 2006; Sasazaki et al., 2006; Jia et al., 2010). This difference may be explained by the number of samples and the haplogroups evaluated. Previous reports used a small number of samples (30 to 108). In addition, the T1 haplogroup and the T1a sub-haplogroup were found in only reports by Jia et al. (2010). Because we used over three times the number of samples, we estimate that our results more accurately reflect the haplogroup frequency in Korean cattle than previous reports.

T1 haplogroup contained only one haplotype and one sample (Table 2). Lei et al. (2006) investigated 231 animals from 20 Chinese native cattle breeds/populations. They reported that the T1 haplogroup was identified in only one animal from the Yanbian and Zaosheng breeds. Kim et al. (2013a, b) reported the phylogenetic relationship between Korean cattle breeds and these the two Chinese breeds. These results suggest that Korean cattle breeds and two Chinese breeds have similar maternal genetic patterns.

All four haplogroups were found in Chikso, Jeju black, and Hanwoo. However, only the T3 and T4 haplogroups appeared in Heugu. This breed is raised only one Management Institute, and the population size is small. Hanwoo was different from the other breeds, with a higher frequency of the T2 haplogroup than the T1 haplogroup. In the Chikso breed, only the CB population contained all haplogroups. However, most Chikso populations showed a partial of four haplogroups. To maintain the genetic diversity of Chikso in the event of various environmental changes, exchange and use of genetic materials among populations is necessary.

In conclusion, we detected sequence variations in the mtDNA control region in Korean cattle breeds, and performed haplogroup distribution at the breed level. These results will be useful for stable conservation and efficient management of Korean cattle breeds. Besides, genetic characteristics based on mtDNA analysis might serve as a source for identification of the maternal origins of cattle, containing Korean cattle breeds.

**CONFLICT OF INTEREST**

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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