

A Thesis

For the Degree of Master of Science in Veterinary Medicine

Analysis of Genetic Polymorphisms and
Phylogenetic tree on mtDNA of Cattle Breeds



Department of Veterinary Medicine

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EunHee Kim

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EunHee Kim

(Supervised by professor YoungMin Yun)

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This thesis has been examined and approved by

Lee KyoungKap

Thesis director, KyoungKap Lee, Prof. of Veterinary Medicine

Shin HyoungDoo

HyoungDoo Shin, Prof. of Life Science, Sogang University

Yun YoungMin

YoungMin Yun, Prof. of Veterinary Medicine

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Analysis of Genetic polymorphisms and Phylogenetic tree on mtDNA of Cattle Breeds

EunHee Kim

(Prof. YoungMin Yun)

*Department of Veterinary Medicine,
Graduate School, Cheju National University*

Abstract

Several researches have been performed to identify mitochondrial DNA(mtDNA) polymorphisms in various breeds of cattle. In this study, we aimed to identify genetic polymorphisms of mtDNA in Korean cattle(*Bos taurus coreana*, Hanwoo) and to analyze genetic relationship with other breeds.

DNAs of 16 Hanwoo and 10 Holstein were amplified using 43 primer sets and direct mtDNA sequencing was performed. For comparison and phylogenetic analysis, complete mtDNA sequences of other breeds, including 7 Japanese black cattle, 2 *Bos indicus*(Nellore, Zwergzebu) and 1 *Bos taurus*(Fleckvieh), were referred to NCBI.

A total, 392 mtDNA polymorphisms were identified including 376 SNPs, 13 Ins/Del and 3 heteroplasmies among breeds. The number of mtDNA polymorphisms within each gene were following; 76 in D loop, 10 in 12s rRNA, 28 in 16s rRNA, 152 in ND1-6, 5 in ND4L, 21 in tRNA, 55 in COX1-3, 8 in ATP 8, 11 in ATP 6, 24 in CYTb, 1 in origin of L-strand replication site, and 1 in intergenic region. Out of 392, 76 polymorphisms were identified in D-loop, which was hypervariable region. Most polymorphisms(355/392) result from transition(A/G or T/C). As a result of phylogenetic analysis with mtDNA polymorphisms, *Bos taurus* breeds(Hanwoo,

Holstein and Japanese black cattle) showed distinct genetic distance from *Bos indicus* breeds(Nellore and Zwergzebu).

The genetic information of mtDNA identified in this study would be useful for further investigation of mtDNA in other breeds.

Key words: mtDNA, polymorphisms, Phylogenetic analysis, Hanwoo, Holstein



I . Introduction

Over 800 breeds of cattle are widely distributed throughout the world. The cattle species are thought to be originated from the aurochs, *Bos primigenius*. *Bos primigenius* has been divided into humpless taurine(*Bos taurus*) and humped zebu(*Bos indicus*) through domestication(Epstein 1971; Epstein and Mason 1984; MacHugh *et al*, 1997).

Bos taurus includes Korean native cattle, Japanese black cattle, Holstein, Jersey, Angus, Limousine, Fleckvieh, *etc.* They are typical breeds of Europe, north-eastern Asia, and parts of Africa and are adapted to cooler climates. *Bos indicus* includes Gir, Guzerat, Kankrej, Indo-Brazilian, Brahman, Nelore, Zwergzebu, *etc.* They are recognized in Africa and South Asia and are adapted to tropical environments than the other domestic cattle.

As mentioned above, various breeds of cattle including the hybrids of two main types(*Bos taurus* and *Bos indicus*) are distributed all over the world. To distinguish between zebu and taurine cattle, several types of genetic markers were used such as protein polymorphisms(Frisch *et al*, 1997), Random Amplified Polymorphic DNAs (RAPDs)(Gwakisa *et al*, 1994; Kemp and Teale, 1994), microsatellites(MacHugh *et al*, 1997) and the mitochondrial DNA variation(Suzuki *et al*, 1993; Loftus *et al*, 1994b; Kikkawa *et al*, 1995; Bradley *et al*, 1996).

Mitochondrion exists in cytoplasm and converts the energy from food into a form that cells can use within cytoplasm. And Mitochondrion has own DNA, called as mitochondrial DNA(mtDNA). The mammalian cells have several thousand copies of double circular mitochondrial mtDNA with 15kb-20kb size(Wolstenholme, 1992;

Boore, 1999). The polymorphisms of mtDNA is high and mtDNA are maternally inherited without recombination.

Following the completion of human mitochondrial DNA sequencing(Anderson *et al*, 1981), the complete mitochondrial genomes of various organisms have been sequenced; *Homo*(Anderson *et al*, 1981; Ozawa *et al*, 1991; Horai *et al*, 1995; Arnason *et al*, 1996), common chimpanzee(Horai *et al*, 1995; Arnason *et al*, 1996), mouse(Bibb *et al*, 1981), the fin(Arnason *et al*, 1991), blue whales(Arnason and Gullberg, 1993) and horse(Xu and Arnason, 1994). Also, the complete 16338 nucleotide DNA sequence of the bovine mitochondrial genome had been determined(Anderson *et al*, 1982). The gene arrangement of bovine mitochondrial genome was identical to human's. The genes contain 12s and 16s ribosomal RNAs, 22 transfer RNAs, and 13 protein components of the inner mitochondrial membrane(Anderson *et al*, 1981). In addition, the genes include more variable non-coding control region(CR) or displacement loop(D-loop) which regulates transcription and replication of the coding DNA strand of the mtDNA(Clayton, 1991).

We aim to investigate the polymorphisms and genetic relationships between Korean native cattle and Holstein and other cattle breeds, through the analysis of complete mtDNA sequences.

II. Materials and Methods

Animals

The animal included Korean Native cattle 16 heads(Hanwoo; HW, male), which were bred in Hanwoo Improvement Center, NACF(National Agricultural Cooperative Federation) and 10 Holstein(HS; male), which were bred in National Livestock Research Institute. To compare their complete mtDNA sequence with other different breeds', sequences for 7 Japanese black cattle, 1 Fleckvieh, 1 Nellore and 1 Zwergzebu were referred to National Center for Biotechnology Information(NCBI) (<http://www.ncbi.nlm.nih.gov/sites/entrez>)(Table 1).

Preparation of DNA and PCR Amplification of complete mtDNA

Total DNA(genomic and mtDNA) was extracted from blood samples of 16 Korean Native Cattle and 10 Holstein by commercial DNA extraction kit(QIAamp DNA blood midi kit, QIAgen[®], USA). The complete mtDNA sequences were amplified by 43 primer sets(Table 2).

Genes of complete mtDNA and full name were presented(Table 3). Also, The structure of mitochondria complete genome of *Bos taurus* was described(Fig.1).

Polymerase chain reaction(PCR) was performed by using the solution of 30 μ l, which consists of sterile distilled water, 10ng of template DNA, 20pmol of each primer, 10Mm of dNTP, 1x buffer and 0.45U of Tag polymerase under touchdown PCR condition, using Thermal cycler(PerkinElmer, USA)(Table 4).

Table 1. Genome information used in this study

Breed		Genome information	Source	
<i>Bos taurus</i>	KNC	Reference	AY526085*(16338 bp)	NCBI
		16 HW	Direct sequencing(16338 bp)	this study
	HS	10 HS	Direct sequencing(16338 bp)	this study
	JBC	7 JBC	AB074968(16337bp)	NCBI
			AB074967(16337bp)	NCBI
			AB074966(16338bp)	NCBI
			AB074965(16337bp)	NCBI
			AB074964(16337bp)	NCBI
			AB074963(16337bp)	NCBI
			AB074962(16337bp)	NCBI
	Fleckvieh	1 Fleck	AF492351(16338bp)	NCBI
<i>Bos indicus</i>	Nellore	1 Nello	AY126697(16341bp)	NCBI
	Zwergzebu	1 Zwer	AF492350(16339bp)	NCBI
Total			36	

The complete mtDNA of 16 HW(*Bos taurus*) and 10 HS(*Bos indicus*) were sequenced directly. To compare with these sequences, other sequences including 7 JBC(*Bos taurus*), 1 Fleckvieh(*Bos taurus*), Nellore(*Bos indicus*) and Zwergzebu(*Bos indicus*) were used.

KNC: Korean Native Cattle, HW: HanWoo, HS: Holstein, JBC: Japanese Black Cattle

*: GeneBank accession number

Table 2. Primer information

No	Name	Forward sequence(5'-3')	Size	Name	Reverse sequence(5'-3')	Size
1	BOSmito-1F	TACAATTCACAGCCAACCC	20	BOSmito-1R	TTGGTGATGACTGTTGCTCC	20
2	BOSmito-2F	GGACGAGGCTTATATTACGGG	21	BOSmito-2R	GCTTCGTTGTTGGAGGTGT	20
3	BOSmito-3F	CGATCAATCCCAACAAACT	20	BOSmito-3R	CCCCTTGCGTAGGTAATTCA	20
4	BOSmito-4F	ACCCCAAAGCTGAAGTTCT	20	BOSmito-4R	CAAGCATCCCCAAAATAAA	20
5	BOSmito-5F	TTCTCAGGGCCATCTCATC	20	BOSmito-5R	GGCTTAATTTTGTCACTGCTG	22
6	BOSmito-6F	GCAAGCATCTACACCCAGT	20	BOSmito-6R	AGCCCATTTCTCCCATTTC	20
7	BOSmito-7F	CTTGGCGGTGCTTTATATCCTT	22	BOSmito-7R	GCGGTACTTTCTCTATAGCGCC	22
8	BOSmito-8F	GCCCAAAGATACCCTCTCGACT	22	BOSmito-8R	TGACTTAGACTTATGCAAGGAGAAAA	26
9	BOSmito-9F	AGTAGGCTAAAAGCAGCCATC	22	BOSmito-9R	TTTTTATTCTCCGAGGTCACCC	22
10	BOSmito-10F	AGGACTTGATGAATGGCCG	20	BOSmito-10R	TTTATGCAATTACCGGGCTC	20
11	BOSmito-11F	TACAATTCACAGCCAACCC	20	BOSmito-11R	TTGGTGATGACTGTTGCTCC	20
12	BOSmito-12F	GGACGAGGCTTATATTACGGG	21	BOSmito-12R	GCTTCGTTGTTGGAGGTGT	20
13	BOSmito-13F	CGATCAATCCCAACAAACT	20	BOSmito-13R	CCCCTTGCGTAGGTAATTCA	20
14	BOSmito-14F	ACCCCAAAGCTGAAGTTCT	20	BOSmito-14R	CAAGCATCCCCAAAATAAA	20
15	BOSmito-15F	TTCTCAGGGCCATCTCATC	20	BOSmito-15R	GGCTTAATTTTGTCACTGCTG	22
16	BOSmito-16F	GCAAGCATCTACACCCAGT	20	BOSmito-16R	AGCCCATTTCTCCCATTTC	20
17	BOSmito-17F	CTTGGCGGTGCTTTATATCCTT	22	BOSmito-17R	GCGGTACTTTCTCTATAGCGCC	22
18	BOSmito-18F	GCCCAAAGATACCCTCTCGACT	22	BOSmito-18R	TGACTTAGACTTATGCAAGGAGAAAA	26
19	BOSmito-19F	AGTAGGCTAAAAGCAGCCATC	22	BOSmito-19R	TTTTTATTCTCCGAGGTCACCC	22
20	BOSmito-20F	AGGACTTGATGAATGGCCG	20	BOSmito-20R	TTTATGCAATTACCGGGCTC	20
21	BOSmito-21F	TGATCTGAGTTCAGACCGGA	20	BOSmito-21R	GGATAATTGCTAGCGTTACTTCG	23
22	BOSmito-22F	CCATGTGAATTCCTACCA	20	BOSmito-22R	ATGCTGTAGTAAGGATGGG	20
23	BOSmito-23F	CCCACAATCCACATACCA	20	BOSmito-23R	AGTTTTATTACGGTTCATTGGC	22
24	BOSmito-24F	TTGCTGAATCGGGTTGAA	20	BOSmito-24R	TAAACCCAGATAGGGGAGGG	20
25	BOSmito-25F	AACCCACCATTAACATTGCT	20	BOSmito-25R	GGCGGAGAAGTAGATTGAA	20
26	BOSmito-26F	CCTTCAAAGCCCTAAGCAAG	20	BOSmito-26R	GAGGATGCGAGGAGTAGTAGGA	22
27	BOSmito-27F	CCGCACACGCATTTGTAATA	20	BOSmito-27R	GGTGGGCTCATACGATGAAA	20
28	BOSmito-28F	GCAGGAGGAGGAGACCCTAT	20	BOSmito-28R	TTCATATTGTGTATGCATCTGGG	23
29	BOSmito-29F	GGGGATTGTTTATTGATTCC	21	BOSmito-29R	TGTATGGGTCAGCTTTGTCTG	20
30	BOSmito-30F	TCCTGTACACCTCATATGGCA	21	BOSmito-30R	TTGACGCAGATCATTTTTCA	20
31	BOSmito-31F	AAAAACAGACGCAATCCAG	20	BOSmito-31R	TGTTGGTGTGAATGAATGGG	20
32	BOSmito-32F	ATTCCCAACATCAAACCGAC	20	BOSmito-32R	GAAATCACATGGTTAGGCCG	20
33	BOSmito-33F	CAGGAGCTTTGTCTGCCCTCT	21	BOSmito-33R	CAAAGCCGAAGTGGTGGTTAG	21
34	BOSmito-34F	CTCTGTCCTATTGGCTTCCG	20	BOSmito-34R	CATGGCAGTGGTAGGAGGAG	20
35	BOSmito-35F	GCCACCCTACTCGTCATCAT	20	BOSmito-35R	GCATTGGAGTAAGTTGAGGTTTT	23
36	BOSmito-36F	AGCAGCCCTAACAAATCCTCA	20	BOSmito-36R	GGGATCCTACTGTGTTTTGGA	21
37	BOSmito-37F	TAACCTTTACCGCCATGGAA	20	BOSmito-37R	GGCTGTGGATTCTTCGTAG	20
38	BOSmito-38F	CGCATACTCTCTGTAAGCCA	21	BOSmito-38R	TTGGTTCTAAGACCAACGG	20
39	BOSmito-39F	TCCTAACCCCTAAACCAAAAA	21	BOSmito-39R	TATGATTCCGACGCTTCTC	20
40	BOSmito-40F	CCCAGTAGCACTATTGTCACA	22	BOSmito-40R	CCAGTTGACTGGATGTGGAG	20
41	BOSmito-41F	CCCTCTGCAATAGAAGGCC	20	BOSmito-41R	GGTTGCTCTAGAAGTGCAGAAAAA	24
42	BOSmito-42F	ATTGCCACCTCTTTCACAGC	20	BOSmito-42R	TGAAGAGGCCATAGGGATTG	20
43	BOSmito-43F	AGCCATCCTACAAAAACCA	20	BOSmito-43R	TCATTAGTCATGGTTAGATTCCATGT	26

The primer of 43 sets were used in this study.

Table 3. Genes and Full name in mtDNA

Gene	Full name	Gene	Full name
D-loop	Displacement-loop	ND1	NADH dehydrogenase subunit 1
12s rRNA	12s ribosomal RNA	ND2	NADH dehydrogenase subunit 2
16s rRNA	16s ribosomal RNA	ND3	NADH dehydrogenase subunit 3
tRNA-Leu	Transfer RNA Leucine	ND4	NADH dehydrogenase subunit 4
tRNA-Met	Transfer RNA Methion	ND4L	NADH dehydrogenase subunit 4L
tRNA-Ala	Transfer RNA Alanine	ND5	NADH dehydrogenase subunit 5
tRNA-Asn	Transfer RNA Asparagine	ND6	NADH dehydrogenase subunit 6
tRNA-Cys	Transfer RNA Cysteine	COX1	Cytochrome oxidase subunit 1
tRNA-Tyr	Transfer RNA Tyrosine	COX2	Cytochrome oxidase subunit 2
tRNA-ASP	Transfer RNA Aspartate	COX3	Cytochrome oxidase subunit 3
tRNA-Gly	Transfer RNA Glycine	ATP 6	ATP synthase subunit 6
tRNA-Ser	Transfer RNA Serine	ATP 8	ATP synthase subunit 8
tRNA-Glu	Transfer RNA Glutamate	CYTb	Cytochrome b
tRNA-Thr	Transfer RNA Threonine	Origin of L-strand replication	-
tRNA-Pro	Transfer RNA Proline	Intergenic	-

Table 4. PCR conditions for mtDNA amplification in each primer set

PCR component	1well(0X)	1well(1X)	1well(1.5X)	1well(2X)
DNA(10ng)	5	5	5	5
Band doctor*(5X)	0	3	6	9
Forward Primer(20pmol)	0.75	0.75	0.75	0.75
Reverse Primer(20pmol)	0.75	0.75	0.75	0.75
10X Buffer	3	3	3	3
dNTP(10mM)	0.6	0.6	0.6	0.6
SolgentTaq®(2.5U/ul)	0.18	0.18	0.18	0.18
DW	19.72	16.72	13.72	10.72
Total	30ul	30ul	30ul	30ul

*: Band doctor[®] was used to get the best quality of PCR products.



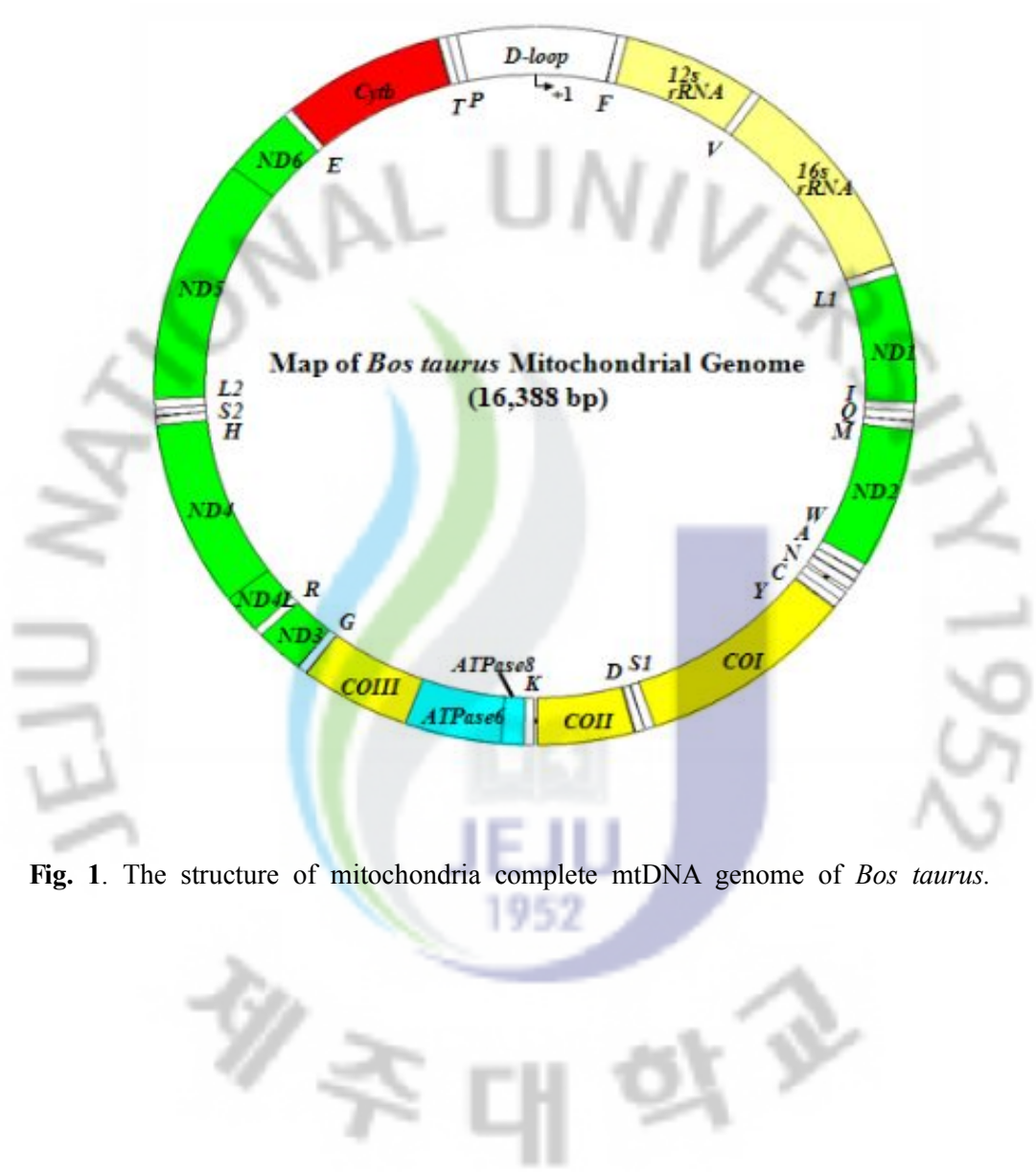


Fig. 1. The structure of mitochondria complete mtDNA genome of *Bos taurus*.

Purification of PCR products and Direct mtDNA Sequencing

PCR products were gel-purified using CleanUp Kit(96 well PCR purification plate, Millipore product). After performing Big Dye[®] process(Table 5), direct mtDNA sequencing was carried out by using the ABI 3700. Multiple alignment mtDNA sequencing was performed by Seqman[®] software(DNASTAR, USA).

Table 5. Big Dye[®] conditions for sequencing of each PCR products

Component	1 tube(ul)
Big Dye(1/8)	1
5X Seq buffer	3
Primer(1pmole)	1.6
Template DNA	2
DW	2.4
Total	10

Data Analysis

For the alignment, the mtDNA sequencing of Hanwoo and Holstein were referred to the mtDNA of Korean Native cattle(GenBank accession no. AY526085). A comparative analysis of minor allele frequency would be performed to investigate polymorphisms and genetic relationships in the sampled breeds. Compared sequences for the analysis were listed in Table 1(<http://www.ncbi.nlm.nih.gov/sites/entrez>).

To show genetic relationship among different cattle breeds, phylogenetic analysis was performed by using biology workbench 2.0(<http://workbench.sdsc.edu>) including complete mtDNA sequences of 36 animals. Also, phylogenetic tree was shown by using representative sequences of each breed. The genetic distance was calculated by using DNADIST including representative sequences of each breed.

III. Results

Complete mtDNA sequences were obtained by direct sequencing in 16 Hanwoo and 10 Holstein. 392 mtDNA polymorphisms of 36 heads including *Bos taurus* and *Bos indicus* were widely distributed over the whole gene(Fig. 2). The information of polymorphisms were listed in table 6 including the loci and frequency of 392 polymorphisms, gene regions, the type of nucleic acid change per each locus, each Minor Allele Frequency(MAF) of three breeds(16 heads of HW, 10 heads of HS and 7 heads of JB) and MAF between two breeds(HW vs JB, HW vs HS, HS vs JB). The SNPID indicate the frequency of 36 cattle including 2 *Bos indicus*(Nellore, Zwergzebu) and 1 Fleckvieh. Thus, in case polymorphism don't exist in any locus of HW, HS or JB, the value of frequency can be Zero.

The number of mtDNA polymorphisms were as follow; 76 in D-Loop, 10 in 12s rRNA, 28 in 16s rRNA, 152 in ND1-6, 5 in ND4L, 21 in tRNA, 55 in COX 1-3, 8 in ATP 8, 11 in ATP 6, 24 in CYTb, 1 in origin of L-strand replication site and 1 in intergenic region. In Hanwoo, 119 polymorphisms were identified; 25 in Holstein, 50 in Japanese black cattle. Much more polymorphisms(76/392) existed in D-Loop than other gene regions.; 35 in Hanwoo, 12 in Japanese black cattle, 17 in Holstein(Table 7).

Of 392 mtDNA polymorphisms, most of nucleic acid change types were the substitution by the transition(The rate of A/G type is 37.2 % and the rate of C/T type is 53.3%. The total rate is 90.5%)(Table 8). 16 Ins/Del types were identified including 2 triallele, 3 heteroplasmies(Table 9).

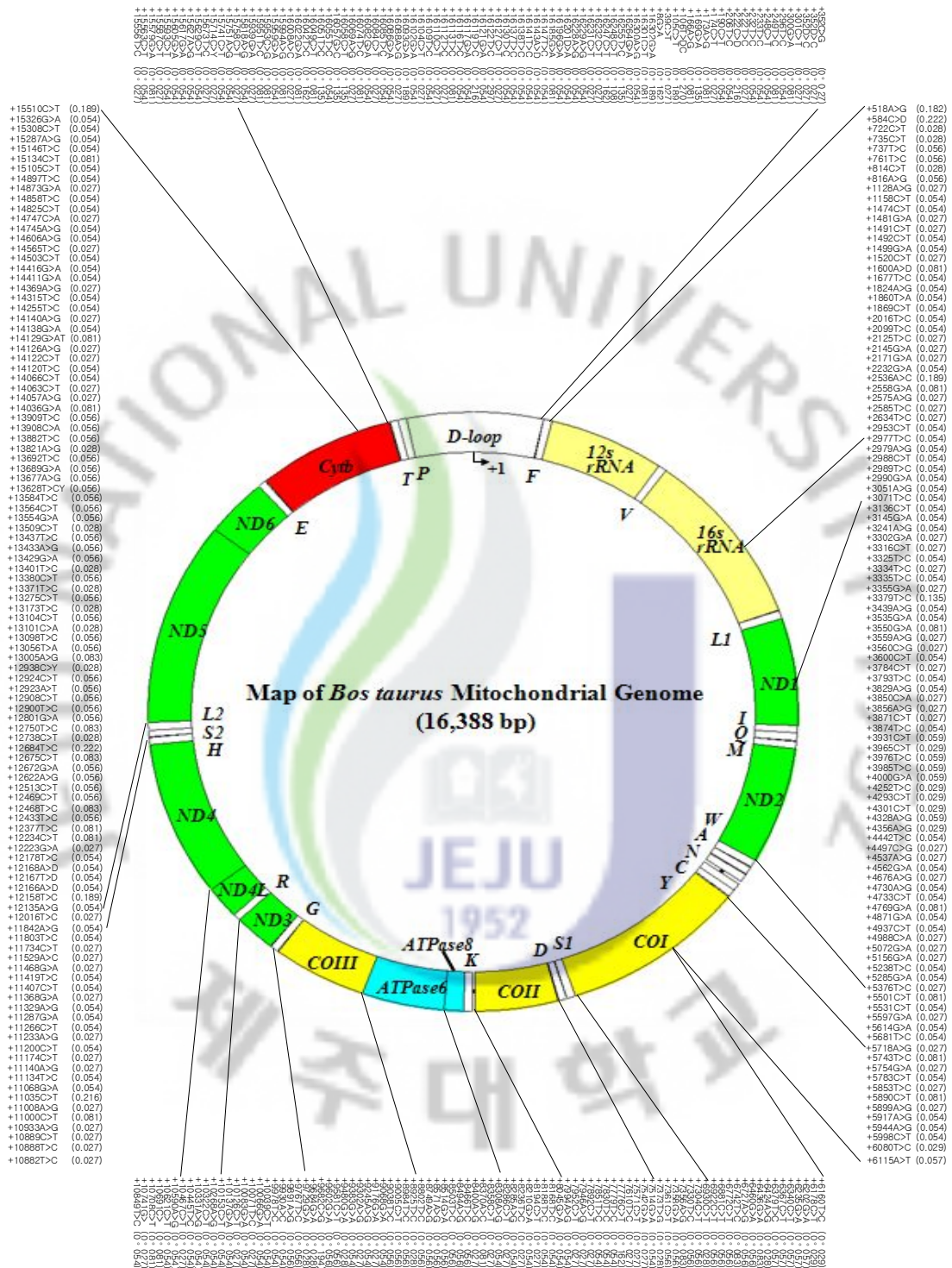


Fig. 2. The map of 392 polymorphisms in mtDNA. 392 polymorphisms were identified in complete mtDNA including 36 heads of cattle.

Table 6. The comparison of polymorphisms in different breeds

SNPID	Gene	Alleles	Minor Allele Frequency			Absolute Difference of MAF		
			HW	HS	JB	HW-JB	HW-HS	HS-JB
+8G>A (0.162)	D-loop	G>A	0.187	0.100	0.000	0.187	0.087	0.100
+39C>T (0.027)	D-loop	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+105C>T (0.189)	D-loop	C>T	0.000	0.000	1.000	1.000	0.000	1.000
+106T>D,C (0.270)	D-loop	T>D,C	0.062	0.000	1.000	0.938	0.062	1.000
+166A>G (0.081)	D-loop	A>G	0.062	0.000	0.000	0.062	0.062	0.000
+169G>A (0.135)	D-loop	G>A	0.062	0.100	0.000	0.062	0.038	0.100
+173A>G (0.081)	D-loop	A>G	0.062	0.100	0.000	0.062	0.038	0.100
+174C>T (0.027)	D-loop	C>T	0.000	0.100	0.000	0.000	0.100	0.100
+190C>T (0.054)	D-loop	C>T	0.062	0.000	0.000	0.062	0.062	0.000
+206T>C (0.054)	D-loop	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+222C>D (0.216)	D-loop	C>D	0.000	0.000	0.857	0.857	0.000	0.857
+222T>C (0.027)	D-loop	T>C	0.062	0.000	0.000	0.062	0.062	0.000
+232T>C (0.054)	D-loop	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+233T>C (0.054)	D-loop	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+248C>T (0.054)	D-loop	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+249T>C (0.081)	D-loop	T>C	0.062	0.000	0.000	0.062	0.062	0.000
+296T>C (0.054)	D-loop	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+300G>A (0.081)	D-loop	G>A	0.062	0.000	0.000	0.062	0.062	0.000
+301C>T (0.027)	D-loop	C>T	0.062	0.000	0.000	0.062	0.062	0.000
+352D>C (0.027)	D-loop	D>C	0.000	0.000	0.000	0.000	0.000	0.000
+352D>C (0.027)	D-loop	D>C	0.000	0.000	0.000	0.000	0.000	0.000
+352C>G (0.027)	D-loop	C>G	0.062	0.000	0.000	0.062	0.062	0.000
+518A>G (0.182)	12s ribosomal RNA	A>G	.	.	0.000	.	.	.
+584C>D (0.222)	12s ribosomal RNA	C>D	0.000	0.000	0.000	0.000	0.000	0.000
+722C>T (0.028)	12s ribosomal RNA	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+735C>T (0.028)	12s ribosomal RNA	C>T	0.000	0.100	0.000	0.000	0.100	0.100
+737T>C (0.056)	12s ribosomal RNA	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+761T>C (0.056)	12s ribosomal RNA	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+814C>T (0.028)	12s ribosomal RNA	C>T	0.067	0.000	0.000	0.067	0.067	0.000
+816A>G (0.056)	12s ribosomal RNA	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+1128A>G (0.027)	12s ribosomal RNA	A>G	0.062	0.000	0.000	0.062	0.062	0.000
+1158C>T (0.054)	12s ribosomal RNA	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+1474C>T (0.054)	16s ribosomal RNA	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+1481G>A (0.027)	16s ribosomal RNA	G>A	0.062	0.000	0.000	0.062	0.062	0.000
+1491C>T (0.027)	16s ribosomal RNA	C>T	0.000	0.100	0.000	0.000	0.100	0.100
+1492C>T (0.054)	16s ribosomal RNA	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+1499G>A (0.054)	16s ribosomal RNA	G>A	0.125	0.000	0.000	0.125	0.125	0.000
+1520C>T (0.027)	16s ribosomal RNA	C>T	0.000	0.100	0.000	0.000	0.100	0.100
+1600A>D (0.081)	16s ribosomal RNA	A>D	0.000	0.000	0.000	0.000	0.000	0.000
+1677T>C (0.054)	16s ribosomal RNA	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+1824A>G (0.054)	16s ribosomal RNA	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+1860T>A (0.054)	16s ribosomal RNA	T>A	0.000	0.000	0.000	0.000	0.000	0.000
+1869C>T (0.054)	16s ribosomal RNA	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+2016T>C (0.054)	16s ribosomal RNA	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+2099T>C (0.054)	16s ribosomal RNA	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+2125T>C (0.027)	16s ribosomal RNA	T>C	0.000	0.100	0.000	0.000	0.100	0.100
+2145G>A (0.027)	16s ribosomal RNA	G>A	0.062	0.000	0.000	0.062	0.062	0.000
+2171G>A (0.027)	16s ribosomal RNA	G>A	0.062	0.000	0.000	0.062	0.062	0.000
+2232G>A (0.054)	16s ribosomal RNA	G>A	0.000	0.000	0.286	0.286	0.000	0.286
+2536A>C (0.189)	16s ribosomal RNA	A>C	0.000	0.000	1.000	1.000	0.000	1.000
+2558G>A (0.081)	16s ribosomal RNA	G>A	0.062	0.000	0.000	0.062	0.062	0.000
+2575A>G (0.027)	16s ribosomal RNA	A>G	0.000	0.000	0.000	0.000	0.000	0.000

Table 6 cont.

SNPID	Gene	Alleles	Minor Allele Frequency			Absolute Difference of MAF		
			HW	HS	JB	HW-JB	HW-HS	HS-JB
+2585T>C (0.027)	16s ribosomal RNA	T>C	0.062	0.000	0.000	0.062	0.062	0.000
+2634T>C (0.027)	16s ribosomal RNA	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+2953C>T (0.054)	16s ribosomal RNA	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+2977T>C (0.054)	16s ribosomal RNA	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+2979A>G (0.054)	16s ribosomal RNA	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+2988C>T (0.054)	16s ribosomal RNA	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+2989T>C (0.054)	16s ribosomal RNA	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+2990G>A (0.054)	16s ribosomal RNA	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+3051A>G (0.054)	tRNA-Leu	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+3071T>C (0.054)	tRNA-Leu	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+3136C>T (0.054)	ND1	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+3145G>A (0.054)	ND1	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+3241A>G (0.054)	ND1	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+3302G>A (0.027)	ND1	G>A	0.062	0.000	0.000	0.062	0.062	0.000
+3316C>T (0.027)	ND1	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+3325T>C (0.054)	ND1	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+3334T>C (0.027)	ND1	T>C	0.000	0.100	0.000	0.000	0.100	0.100
+3335T>C (0.054)	ND1	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+3355G>A (0.027)	ND1	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+3379T>C (0.135)	ND1	T>C	0.187	0.000	0.000	0.187	0.187	0.000
+3439A>G (0.054)	ND1	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+3535G>A (0.054)	ND1	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+3550G>A (0.081)	ND1	G>A	0.062	0.000	0.000	0.062	0.062	0.000
+3559A>G (0.027)	ND1	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+3560C>G (0.027)	ND1	C>G	0.000	0.000	0.000	0.000	0.000	0.000
+3600C>T (0.054)	ND1	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+3784C>T (0.027)	ND1	C>T	0.000	0.100	0.000	0.000	0.100	0.100
+3793T>C (0.054)	ND1	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+3829A>G (0.054)	ND1	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+3850C>A (0.027)	ND1	C>A	0.000	0.100	0.000	0.000	0.100	0.100
+3856A>G (0.027)	ND1	A>G	0.062	0.000	0.000	0.062	0.062	0.000
+3871C>T (0.027)	ND1	C>T	0.062	0.000	0.000	0.062	0.062	0.000
+3874T>C (0.054)	ND1	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+3931C>T (0.059)	ND1	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+3965C>T (0.029)	ND1	C>T	0.062	0.000	0.000	0.062	0.062	0.000
+3976T>C (0.059)	ND1	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+3985T>C (0.059)	ND1	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+4000G>A (0.059)	ND1	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+4252T>C (0.029)	tRNA-Met	T>C	0.062	0.000	0.000	0.062	0.062	0.000
+4293C>T (0.029)	ND2	C>T	0.062	0.000	0.000	0.062	0.062	0.000
+4301C>T (0.029)	ND2	C>T	0.000	0.000	0.143	0.143	0.000	0.143
+4328A>G (0.059)	ND2	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+4356A>G (0.029)	ND2	A>G	0.062	0.000	0.000	0.062	0.062	0.000
+4442T>C (0.054)	ND2	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+4497C>G (0.027)	ND2	C>G	0.000	0.000	0.000	0.000	0.000	0.000
+4537A>G (0.027)	ND2	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+4562G>A (0.054)	ND2	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+4676A>G (0.027)	ND2	A>G	0.062	0.000	0.000	0.062	0.062	0.000
+4730A>G (0.054)	ND2	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+4733C>T (0.054)	ND2	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+4769G>A (0.081)	ND2	G>A	0.000	0.100	0.000	0.000	0.100	0.100
+4871G>A (0.054)	ND2	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+4937C>T (0.054)	ND2	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+4988C>A (0.027)	ND2	C>A	0.000	0.000	0.000	0.000	0.000	0.000

Table 6 cont.

SNPID	Gene	Alleles	Minor Allele Frequency			Absolute Difference of MAF		
			HW	HS	JB	HW-JB	HW-HS	HS-JB
+5072G>A (0.027)	ND2	G>A	0.000	0.100	0.000	0.000	0.100	0.100
+5156G>A (0.027)	ND2	G>A	0.062	0.000	0.000	0.062	0.062	0.000
+5238T>C (0.054)	ND2	T>C	0.125	0.000	0.000	0.125	0.125	0.000
+5285G>A (0.054)	ND2	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+5376T>C (0.027)	tRNA-Ala	T>C	0.062	0.000	0.000	0.062	0.062	0.000
+5501C>T (0.081)	tRNA-Asn	C>T	0.062	0.000	0.000	0.062	0.062	0.000
+5531C>T (0.054)	origin of L-strand replication	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+5597G>A (0.027)	tRNA-Cys	G>A	0.062	0.000	0.000	0.062	0.062	0.000
+5614G>A (0.054)	tRNA-Cys	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+5681T>C (0.054)	tRNA-Tyr	T>C	0.125	0.000	0.000	0.125	0.125	0.000
+5718A>G (0.027)	cox1	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+5743T>C (0.081)	cox1	T>C	0.062	0.000	0.000	0.062	0.062	0.000
+5754G>A (0.027)	cox1	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+5783C>T (0.054)	cox1	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+5853T>C (0.027)	cox1	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+5890C>T (0.081)	cox1	C>T	0.062	0.000	0.000	0.062	0.062	0.000
+5899A>G (0.027)	cox1	A>G	0.062	0.000	0.000	0.062	0.062	0.000
+5917A>G (0.054)	cox1	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+5944A>G (0.054)	cox1	A>G	0.125	0.000	0.000	0.125	0.125	0.000
+5998C>T (0.054)	cox1	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+6080T>C (0.029)	cox1	T>C	0.000	0.100	0.000	0.000	0.100	0.100
+6115A>T (0.057)	cox1	A>T	0.000	0.000	0.000	0.000	0.000	0.000
+6160T>C (0.029)	cox1	T>C	0.071	0.000	0.000	0.071	0.071	0.000
+6178C>T (0.029)	cox1	C>T	0.000	0.000	0.143	0.143	0.000	0.143
+6202G>A (0.057)	cox1	G>A	0.071	0.100	0.000	0.071	0.029	0.100
+6235G>A (0.057)	cox1	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+6340C>T (0.057)	cox1	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+6367C>T (0.057)	cox1	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+6379T>C (0.057)	cox1	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+6424A>G (0.028)	cox1	A>G	0.000	0.000	0.143	0.143	0.000	0.143
+6436G>A (0.083)	cox1	G>A	0.067	0.000	0.000	0.067	0.067	0.000
+6460G>A (0.056)	cox1	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+6727A>G (0.056)	cox1	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+6742T>C (0.083)	cox1	T>C	0.133	0.100	0.000	0.133	0.033	0.100
+6772C>T (0.056)	cox1	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+6881C>T (0.056)	cox1	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+6922C>T (0.056)	cox1	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+6930C>T (0.028)	cox1	C>T	0.000	0.100	0.000	0.000	0.100	0.100
+7304C>T (0.056)	tRNA-ASP	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+7330T>C (0.056)	tRNA-ASP	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+7356A>G (0.083)	tRNA-ASP	A>G	0.062	0.000	0.000	0.062	0.062	0.000
+7358G>A (0.056)	tRNA-ASP	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+7361C>T (0.056)	tRNA-ASP	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+7373T>G (0.028)	intergenic	T>G	0.062	0.000	0.000	0.062	0.062	0.000
+7514G>A (0.054)	cox2	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+7542G>A (0.027)	cox2	G>A	0.062	0.000	0.000	0.062	0.062	0.000
+7571C>T (0.027)	cox2	C>T	0.000	0.100	0.000	0.000	0.100	0.100
+7610T>C (0.027)	cox2	T>C	0.000	0.100	0.000	0.000	0.100	0.100
+7776C>T (0.162)	cox2	C>T	0.250	0.200	0.000	0.250	0.050	0.200
+7779T>C (0.054)	cox2	T>C	0.125	0.000	0.000	0.125	0.125	0.000
+7830T>C (0.054)	cox2	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+7851T>C (0.054)	cox2	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+7892C>T (0.027)	cox2	C>T	0.000	0.000	0.000	0.000	0.000	0.000

Table 6 cont.

SNPID	Gene	Alleles	Minor Allele Frequency			Absolute Difference of MAF		
			HW	HS	JB	HW-JB	HW-HS	HS-JB
+7946A>G (0.027)	cox2	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+7952C>T (0.027)	cox2	C>T	0.062	0.000	0.000	0.062	0.062	0.000
+7994A>G (0.054)	cox2	A>G	0.062	0.000	0.000	0.062	0.062	0.000
+8045G>A (0.054)	cox2	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+8168T>C (0.054)	ATP8	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+8188T>C (0.054)	ATP8	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+8194C>T (0.027)	ATP8	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+8210G>A (0.054)	ATP8	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+8236T>C (0.027)	ATP8	T>C	0.062	0.000	0.000	0.062	0.062	0.000
+8285A>G (0.054)	ATP8	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+8286C>T (0.027)	ATP8	C>T	0.000	0.100	0.000	0.000	0.100	0.100
+8308A>G (0.054)	ATP8	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+8358C>T (0.027)	ATP6	C>T	0.062	0.000	0.000	0.062	0.062	0.000
+8370A>C (0.081)	ATP6	A>C	0.062	0.000	0.000	0.062	0.062	0.000
+8400A>G (0.027)	ATP6	A>G	0.000	0.000	0.143	0.143	0.000	0.143
+8466T>C (0.056)	ATP6	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+8494A>G (0.056)	ATP6	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+8503T>C (0.056)	ATP6	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+8514G>A (0.056)	ATP6	G>A	0.000	0.111	0.000	0.000	0.111	0.111
+8571A>G (0.056)	ATP6	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+8749A>G (0.056)	ATP6	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+8802T>C (0.056)	ATP6	T>C	0.125	0.000	0.000	0.125	0.125	0.000
+8925T>C (0.028)	ATP6	T>C	0.062	0.000	0.000	0.062	0.062	0.000
+8984T>C (0.056)	COX3	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+9005C>T (0.056)	COX3	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+9038T>C (0.056)	COX3	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+9068G>A (0.054)	COX3	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+9176G>A (0.027)	COX3	G>A	0.000	0.100	0.000	0.000	0.100	0.100
+9245A>G (0.054)	COX3	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+9302A>G (0.027)	COX3	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+9383G>A (0.054)	COX3	G>A	0.125	0.000	0.000	0.125	0.125	0.000
+9480G>A (0.028)	COX3	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+9581C>T (0.056)	COX3	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+9602G>A (0.056)	COX3	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+9682C>G (0.194)	COX3	C>G	0.000	0.000	1.000	1.000	0.000	1.000
+9684G>A (0.028)	COX3	G>A	0.000	0.100	0.000	0.000	0.100	0.100
+9729G>A (0.028)	COX3	G>A	0.000	0.100	0.000	0.000	0.100	0.100
+9767T>C (0.056)	tRNA-Gly	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+9891A>G (0.056)	ND3	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+9930A>C (0.054)	ND3	A>C	0.000	0.000	0.000	0.000	0.000	0.000
+9978T>C (0.054)	ND3	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+10039C>T (0.054)	ND3	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+10066G>A (0.054)	ND3	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+10071T>C (0.027)	ND3	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+10083G>A (0.054)	ND3	G>A	0.125	0.000	0.000	0.125	0.125	0.000
+10126C>T (0.027)	ND3	C>T	0.062	0.000	0.000	0.062	0.062	0.000
+10137G>A (0.054)	ND3	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+10153C>T (0.054)	ND3	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+10268A>G (0.054)	ND4L	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+10322C>T (0.054)	ND4L	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+10331A>G (0.054)	ND4L	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+10445T>C (0.054)	ND4L	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+10467C>T (0.027)	ND4L	C>T	0.062	0.000	0.000	0.062	0.062	0.000
+10590A>G (0.054)	ND4	A>G	0.000	0.000	0.000	0.000	0.000	0.000

Table 6 cont.

SNPID	Gene	Alleles	Minor Allele Frequency			Absolute Difference of MAF		
			HW	HS	JB	HW-JB	HW-HS	HS-JB
+10621C>T (0.054)	ND4	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+10691C>G (0.081)	ND4	C>G	0.062	0.000	0.000	0.062	0.062	0.000
+10708C>T (0.081)	ND4	C>T	0.125	0.100	0.000	0.125	0.025	0.100
+10741G>A (0.027)	ND4	G>A	0.062	0.000	0.000	0.062	0.062	0.000
+10849T>C (0.054)	ND4	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+10882T>C (0.027)	ND4	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+10888T>C (0.027)	ND4	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+10889C>T (0.027)	ND4	C>T	0.000	0.000	0.143	0.143	0.000	0.143
+10933A>G (0.027)	ND4	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+11000C>T (0.081)	ND4	C>T	0.062	0.000	0.000	0.062	0.062	0.000
+11008A>G (0.027)	ND4	A>G	0.062	0.000	0.000	0.062	0.062	0.000
+11035C>T (0.216)	ND4	C>T	0.250	0.200	0.000	0.250	0.050	0.200
+11068G>A (0.054)	ND4	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+11134T>C (0.054)	ND4	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+11140A>G (0.027)	ND4	A>G	0.062	0.000	0.000	0.062	0.062	0.000
+11174C>T (0.027)	ND4	C>T	0.000	0.000	0.143	0.143	0.000	0.143
+11200C>T (0.054)	ND4	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+11233A>G (0.027)	ND4	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+11266C>T (0.054)	ND4	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+11287G>A (0.054)	ND4	G>A	0.125	0.000	0.000	0.125	0.125	0.000
+11329A>G (0.054)	ND4	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+11368G>A (0.027)	ND4	G>A	0.000	0.100	0.000	0.000	0.100	0.100
+11407C>T (0.054)	ND4	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+11419T>C (0.054)	ND4	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+11468G>A (0.027)	ND4	G>A	0.062	0.000	0.000	0.062	0.062	0.000
+11529A>C (0.027)	ND4	A>C	0.000	0.100	0.000	0.000	0.100	0.100
+11734C>T (0.027)	ND4	C>T	0.000	0.100	0.000	0.000	0.100	0.100
+11803T>C (0.054)	ND4	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+11842A>G (0.054)	ND4	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+12016T>C (0.027)	tRNA-Ser	T>C	0.062	0.000	0.000	0.062	0.062	0.000
+12135A>G (0.054)	ND5	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+12158T>C (0.189)	ND5	T>C	0.187	0.000	0.429	0.242	0.187	0.429
+12166A>D (0.054)	ND5	A>D	0.125	0.000	0.000	0.125	0.125	0.000
+12167T>D (0.054)	ND5	T>D	0.125	0.000	0.000	0.125	0.125	0.000
+12168A>D (0.054)	ND5	A>D	0.125	0.000	0.000	0.125	0.125	0.000
+12178T>C (0.054)	ND5	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+12223G>A (0.027)	ND5	G>A	0.000	0.100	0.000	0.000	0.100	0.100
+12234C>T (0.081)	ND5	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+12377T>C (0.081)	ND5	T>C	0.062	0.000	0.000	0.062	0.062	0.000
+12433T>C (0.056)	ND5	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+12468T>C (0.083)	ND5	T>C	0.067	0.000	0.000	0.067	0.067	0.000
+12469C>T (0.056)	ND5	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+12513C>T (0.056)	ND5	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+12622A>G (0.056)	ND5	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+12672G>A (0.056)	ND5	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+12675C>T (0.083)	ND5	C>T	0.067	0.000	0.000	0.067	0.067	0.000
+12684T>C (0.222)	ND5	T>C	0.267	0.200	0.000	0.267	0.067	0.200
+12738C>T (0.028)	ND5	C>T	0.067	0.000	0.000	0.067	0.067	0.000
+12750T>C (0.083)	ND5	T>C	0.067	0.000	0.000	0.067	0.067	0.000
+12801G>A (0.056)	ND5	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+12900T>C (0.056)	ND5	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+12908C>T (0.056)	ND5	C>T	0.000	0.000	0.286	0.286	0.000	0.286
+12923A>T (0.056)	ND5	A>T	0.000	0.000	0.000	0.000	0.000	0.000
+12924C>T (0.056)	ND5	C>T	0.000	0.000	0.000	0.000	0.000	0.000

Table 6 cont.

SNPID	Gene	Alleles	Minor Allele Frequency			Absolute Difference of MAF		
			HW	HS	JB	HW-JB	HW-HS	HS-JB
+12938C>Y (0.028)	ND5	C>Y	0.067	0.000	0.000	0.067	0.067	0.000
+13005A>G (0.083)	ND5	A>G	0.062	0.000	0.000	0.062	0.062	0.000
+13056T>A (0.056)	ND5	T>A	0.000	0.000	0.000	0.000	0.000	0.000
+13098T>C (0.056)	ND5	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+13101C>A (0.028)	ND5	C>A	0.000	0.000	0.000	0.000	0.000	0.000
+13104C>T (0.056)	ND5	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+13173T>C (0.028)	ND5	T>C	0.000	0.111	0.000	0.000	0.111	0.111
+13275C>T (0.056)	ND5	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+13371T>C (0.028)	ND5	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+13380C>T (0.056)	ND5	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+13401T>C (0.028)	ND5	T>C	0.067	0.000	0.000	0.067	0.067	0.000
+13429G>A (0.056)	ND5	G>A	0.133	0.000	0.000	0.133	0.133	0.000
+13433A>G (0.056)	ND5	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+13437T>C (0.056)	ND5	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+13509C>T (0.028)	ND5	C>T	0.067	0.000	0.000	0.067	0.067	0.000
+13554G>A (0.056)	ND5	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+13564C>T (0.056)	ND5	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+13584T>C (0.056)	ND5	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+13628T>C,Y (0.056)	ND5	T>C,Y	0.000	0.100	0.000	0.000	0.100	0.100
+13677A>G (0.056)	ND5	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+13689G>A (0.056)	ND5	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+13692T>C (0.056)	ND5	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+13821A>G (0.028)	ND5	A>G	0.067	0.000	0.000	0.067	0.067	0.000
+13882T>C (0.056)	ND5	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+13908C>A (0.056)	ND5	C>A	0.000	0.000	0.000	0.000	0.000	0.000
+13909T>C (0.056)	ND5	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+14036G>A (0.081)	ND6	G>A	0.062	0.000	0.000	0.062	0.062	0.000
+14057A>G (0.027)	ND6	A>G	0.062	0.000	0.000	0.062	0.062	0.000
+14063C>T (0.027)	ND6	C>T	0.000	0.100	0.000	0.000	0.100	0.100
+14066C>T (0.054)	ND6	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+14120T>C (0.054)	ND6	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+14122C>T (0.027)	ND6	C>T	0.000	0.000	0.143	0.143	0.000	0.143
+14126A>G (0.027)	ND6	A>G	0.000	0.100	0.000	0.000	0.100	0.100
+14129G>A,T (0.081)	ND6	G>A,T	0.062	0.000	0.000	0.062	0.062	0.000
+14138G>A (0.054)	ND6	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+14140A>G (0.027)	ND6	A>G	0.000	0.000	0.143	0.143	0.000	0.143
+14255T>C (0.054)	ND6	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+14315T>C (0.054)	ND6	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+14369A>G (0.027)	ND6	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+14411G>A (0.054)	ND6	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+14416G>A (0.054)	ND6	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+14503C>T (0.054)	tRNA-Glu	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+14565T>C (0.027)	CYTB	T>C	0.000	0.000	0.143	0.143	0.000	0.143
+14606A>G (0.054)	CYTB	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+14745A>G (0.054)	CYTB	A>G	0.125	0.000	0.000	0.125	0.125	0.000
+14747C>A (0.027)	CYTB	C>A	0.062	0.000	0.000	0.062	0.062	0.000
+14825C>T (0.054)	CYTB	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+14858T>C (0.054)	CYTB	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+14873G>A (0.027)	CYTB	G>A	0.062	0.000	0.000	0.062	0.062	0.000
+14897T>C (0.054)	CYTB	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+15105C>T (0.054)	CYTB	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+15134C>T (0.081)	CYTB	C>T	0.062	0.000	0.000	0.062	0.062	0.000
+15146T>C (0.054)	CYTB	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+15287A>G (0.054)	CYTB	A>G	0.000	0.000	0.000	0.000	0.000	0.000

Table 6 cont.

SNPID	Gene	Alleles	Minor Allele Frequency			Absolute Difference of MAF		
			HW	HS	JB	HW-JB	HW-HS	HS-JB
+15308C>T (0.054)	CYTB	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+15326G>A (0.054)	CYTB	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+15510C>T (0.189)	CYTB	C>T	0.187	0.000	0.429	0.242	0.187	0.429
+15556T>C (0.027)	CYTB	T>C	0.000	0.100	0.000	0.000	0.100	0.100
+15563C>T (0.054)	CYTB	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+15579G>A (0.081)	CYTB	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+15587C>T (0.027)	CYTB	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+15593C>T (0.054)	CYTB	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+15605G>A (0.054)	CYTB	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+15617G>A (0.054)	CYTB	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+15627A>G (0.054)	CYTB	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+15629C>T (0.054)	CYTB	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+15673T>C (0.027)	tRNA- Thr	T>C	0.062	0.000	0.000	0.062	0.062	0.000
+15714C>Y (0.027)	tRNA- Thr	C>Y	0.062	0.000	0.000	0.062	0.062	0.000
+15741C>T (0.054)	tRNA- Pro	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+15751A>G (0.054)	tRNA- Pro	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+15758C>T (0.027)	tRNA- Pro	C>T	0.062	0.000	0.000	0.062	0.062	0.000
+15818A>G (0.054)	D-loop	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+15939G>A (0.027)	D-loop	G>A	0.000	0.100	0.000	0.000	0.100	0.100
+15951T>C (0.081)	D-loop	T>C	0.062	0.000	0.000	0.062	0.062	0.000
+15953C>G (0.081)	D-loop	C>G	0.062	0.000	0.000	0.062	0.062	0.000
+15959G>A (0.054)	D-loop	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+15994A>G (0.081)	D-loop	A>G	0.062	0.000	0.000	0.062	0.062	0.000
+16008A>C (0.027)	D-loop	A>C	0.000	0.100	0.000	0.000	0.100	0.100
+16022G>A (0.081)	D-loop	G>A	0.062	0.000	0.000	0.062	0.062	0.000
+16042T>C (0.162)	D-loop	T>C	0.187	0.000	0.286	0.099	0.187	0.286
+16049C>T (0.081)	D-loop	C>T	0.062	0.000	0.000	0.062	0.062	0.000
+16051T>C (0.135)	D-loop	T>C	0.250	0.100	0.000	0.250	0.150	0.100
+16055T>C (0.054)	D-loop	T>C	0.000	0.000	0.286	0.286	0.000	0.286
+16057G>C,A (0.135)	D-loop	G>C,A	0.125	0.100	0.000	0.125	0.025	0.100
+16058C>T (0.135)	D-loop	C>T	0.062	0.100	0.143	0.081	0.038	0.043
+16069A>G (0.027)	D-loop	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+16074T>C (0.081)	D-loop	T>C	0.062	0.000	0.000	0.062	0.062	0.000
+16082G>A (0.054)	D-loop	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+16084C>T (0.027)	D-loop	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+16085T>C (0.054)	D-loop	T>C	0.062	0.000	0.000	0.062	0.062	0.000
+16086G>A (0.054)	D-loop	G>A	0.125	0.000	0.000	0.125	0.125	0.000
+16088A>G (0.027)	D-loop	A>G	0.000	0.000	0.143	0.143	0.000	0.143
+16093G>A (0.189)	D-loop	G>A	0.187	0.000	0.429	0.242	0.187	0.429
+16102G>A (0.054)	D-loop	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+16104C>T (0.027)	D-loop	C>T	0.000	0.000	0.143	0.143	0.000	0.143
+16109T>C (0.054)	D-loop	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+16110C>T (0.027)	D-loop	C>T	0.000	0.100	0.000	0.000	0.100	0.100
+16112T>C (0.027)	D-loop	T>C	0.000	0.000	0.143	0.143	0.000	0.143
+16113T>C (0.081)	D-loop	T>C	0.062	0.000	0.000	0.062	0.062	0.000
+16116T>C (0.054)	D-loop	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+16117G>A (0.054)	D-loop	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+16119T>C (0.216)	D-loop	T>C	0.125	0.200	0.286	0.161	0.075	0.086
+16121G>A (0.054)	D-loop	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+16122T>C (0.297)	D-loop	T>C	0.312	0.300	0.143	0.169	0.012	0.157
+16127C>T (0.027)	D-loop	C>T	0.000	0.100	0.000	0.000	0.100	0.100
+16130T>C (0.054)	D-loop	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+16137T>C (0.054)	D-loop	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+16138T>C (0.054)	D-loop	T>C	0.000	0.000	0.000	0.000	0.000	0.000

Table 6 cont.

SNPID	Gene	Alleles	Minor Allele Frequency			Absolute Difference of MAF		
			HW	HS	JB	HW-JB	HW-HS	HS-JB
+16141T>C (0.054)	D-loop	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+16143A>D (0.054)	D-loop	A>D	0.000	0.000	0.000	0.000	0.000	0.000
+16147T>C (0.054)	D-loop	T>C	0.000	0.000	0.000	0.000	0.000	0.000
+16185G>A (0.081)	D-loop	G>A	0.125	0.100	0.000	0.125	0.025	0.100
+16196G>A (0.054)	D-loop	G>A	0.000	0.000	0.000	0.000	0.000	0.000
+16201D>A (0.054)	D-loop	D>A	0.000	0.000	0.000	0.000	0.000	0.000
+16228A>G (0.054)	D-loop	A>G	0.125	0.000	0.000	0.125	0.125	0.000
+16229A>G (0.054)	D-loop	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+16231C>T (0.027)	D-loop	C>T	0.062	0.000	0.000	0.062	0.062	0.000
+16232C>T (0.027)	D-loop	C>T	0.000	0.000	0.000	0.000	0.000	0.000
+16247C>T (0.162)	D-loop	C>T	0.250	0.000	0.000	0.250	0.250	0.000
+16248C>T (0.108)	D-loop	C>T	0.062	0.100	0.000	0.062	0.038	0.100
+16255T>C (0.135)	D-loop	T>C	0.250	0.100	0.000	0.250	0.150	0.100
+16264G>A (0.027)	D-loop	G>A	0.062	0.000	0.000	0.062	0.062	0.000
+16300A>G (0.054)	D-loop	A>G	0.000	0.000	0.000	0.000	0.000	0.000
+16301C>T (0.081)	D-loop	C>T	0.000	0.100	0.000	0.000	0.100	0.100
+16302G>A (0.189)	D-loop	G>A	0.187	0.000	0.571	0.384	0.187	0.571

A: Adenin, T: Tymin G: Guanine C: Cytocine

SNPID indicate the type of nucleic acid change and frequency of 392 polymorphism including 36 cattle. Thus even though polymorphism are indicated in SNPID, the value of frequency can be zero in HW, HS or JB if any polymorphism don't exist in locus of HW, HS or JB.

HW-JB:HW-HS:HS-JB: The value means absolute difference of minor allele frequency between two breeds.

Table 7. Genes and Number of polymorphisms in 36 cattle(*Bos taurus* and *Bos indicus*)

Gene	Full name	Number of all polymorphisms	Number of each breed polymorphisms		
			16 heads of HW	7 heads of JB	16 heads of HS
D-loop		76	35	12	17
12s rRNA	12s ribosomal RNA	10	2	0	1
16s rRNA	16s ribosomal RNA	28	6	1	3
tRNA-Leu	Transfer RNA Leucine	2	0	0	0
tRNA-Met	Transfer RNA Methion	1	0	0	0
tRNA-Ala	Transfer RNA Alanine	1	1	0	0
tRNA-Asn	Transfer RNA Asparagine	1	1	0	0
tRNA-Cys	Transfer RNA Cysteine	2	1	0	0
tRNA-Tyr	Transfer RNA Tyrosine	1	1	0	0
tRNA-ASP	Transfer RNA Aspartate	5	1	0	0
tRNA-Gly	Transfer RNA Glycine	1	0	0	0
tRNA-Ser	Transfer RNA Serine	1	1	0	0
tRNA-Glu	Transfer RNA Glutamate	1	0	0	0
tRNA-Thr	Transfer RNA Threonine	2	2	0	0
tRNA-Pro	Transfer RNA Proline	3	1	0	0
ND1	NADH dehydrogenase subunit 1	28	6	0	3
ND2	NADH dehydrogenase subunit 2	19	5	1	2
ND3	NADH dehydrogenase subunit 3	10	2	0	0
ND4	NADH dehydrogenase subunit 4	30	9	2	5
ND4L	NADH dehydrogenase subunit 4L	5	1	0	0
ND5	NADH dehydrogenase subunit 5	50	16	2	4
ND6	NADH dehydrogenase subunit 6	15	3	2	2
COX1	Cytochrome coxidase subunit 1	28	8	2	4
COX2	Cytochrome coxidase subunit 2	13	5	0	3
COX3	Cytochrome coxidase subunit 3	14	1	0	3
ATP 6	ATP synthase subunit 6	11	4	1	1
ATP 8	ATP synthase subunit 8	8	1	0	1
CYTb	Cytochrome b	24	5	2	1
Origin of L-strand replication	-	1	0	0	0
Intergenic	-	1	1	0	0
Total	-	392	119	25	50

Table 8. The type of nucleic acid substitutions in mtDNA polymorphisms of 36 cattle

Type of nucleic acid substitutions	Number	Rate(%)
A/G	146	37.2
C/T	209	53.3
A/C	10	2.6
G/T	1	0.3
C/G	6	1.5
A/T	4	1.0
Others	16	4.1
Total	392	100

* Most nucleic acid substitutions occurred through transitions.
(A/G is 146/392, C/T is 209/392)

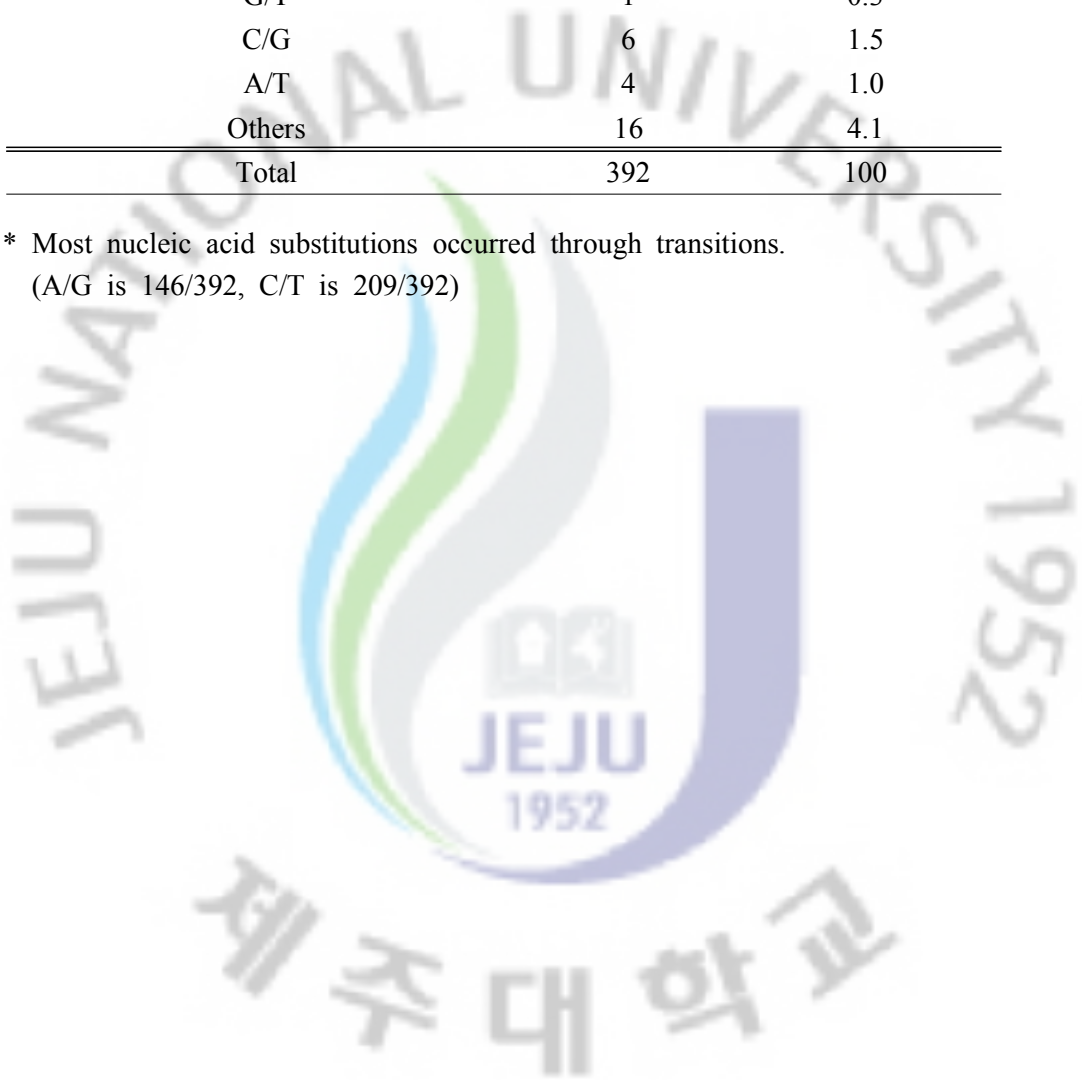


Table 9. The Ins/Del type of polymorphisms in mtDNA of 36 cattle

SNPID2	Position	Gene	Minor Freq.	Type	Type of event
+1600A>D*	1600	16s ribosomal RNA	0.081	A/D	Deletion
+12166A>D (0.054)	12166	ND5	0.054	A/D	Deletion
+12168A>D (0.054)	12168	ND5	0.054	A/D	Deletion
+16143A>D (0.054)	16143	D-loop	0.054	A/D	Deletion
+584C>D (0.222)	584	12s ribosomal RNA	0.222	C/D	Deletion
+222C>D (0.216)	222	D-loop	0.216	C/D	Deletion
+16201D>A (0.054)	1620	D-loop	0.054	D/A	Insertion
+352D>C (0.027)	352	D-loop	0.027	D/C	Deletion
+352D>C (0.027)	352	D-loop	0.027	D/C	Insertion
+12167T>D (0.054)	12167	ND5	0.054	T/D	Deletion
+106T>D,C (0.270)	106	D-loop	0.270	TD,C	Deletion&Substitution
+14129G>A,T(0.081)	14129	ND6	0.081	G>A,T	Triallele
+16057G>C,A(0.135)	16057	D-loop	0.135	G>C,A	Triallele
+13628T>C,Y(0.056)	13628	ND5	0.056	T>C,Y**	Heteroplasmy
+15714C>Y (0.027)	15714	tRNA- Thr	0.027	C/Y	Heteroplasmy
+15714C>Y (0.027)	15714	tRNA- Thr	0.027	C/Y	Heteroplasmy

*D: A, G, T **Y: C, T

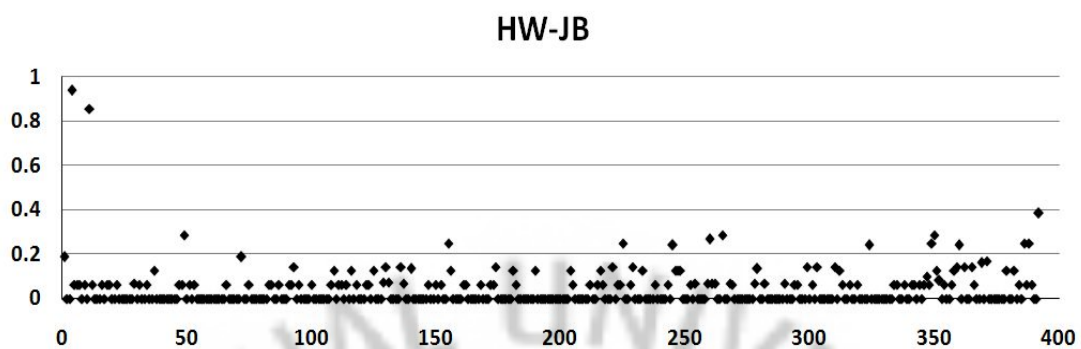


Fig. 3. Difference of minor allele frequency between Hanwoo and Japanese black cattle

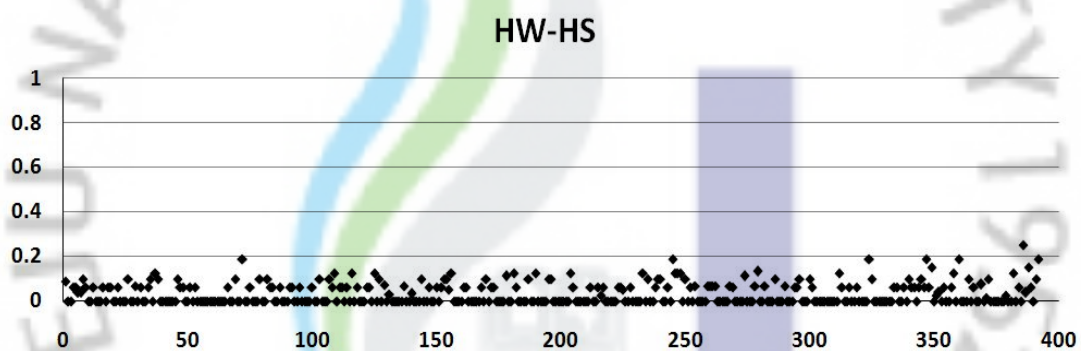


Fig. 4. Difference of minor allele frequency between Hanwoo and Holstein

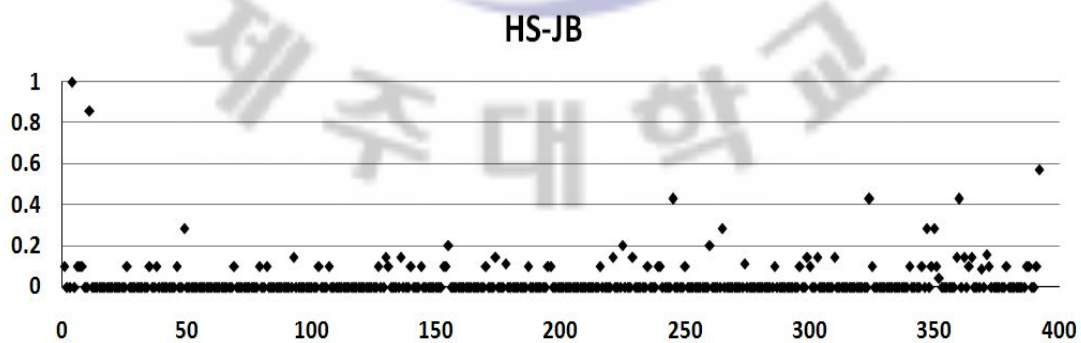


Fig. 5. Difference of minor allele frequency between Holstein and Japanese black cattle

There has been low difference of minor allele frequency between Hanwoo and Holstein than other case(HW vs JB, HS vs JB)(Fig. 3, Fig. 4 and Fig. 5). Difference of minor allele frequency more than 0.2 were listed in table 10(HW vs JB), table 11(HW vs HS) and table 12(HS vs JB).



Table 10. Difference of minor allele frequency between HW and JB more than 0.2

SNPID2	Gene	HW-JB
+106T>D,C(0.270)	D-loop	0.938
+222C>D(0.216)	D-loop	0.857
+16302G>A(0.189)	D-loop	0.384
+2232G>A(0.054)	16s ribosomal RNA	0.286
+12908C>T(0.056)	ND5	0.286
+16055T>C(0.054)	D-loop	0.286
+12684T>C(0.222)	ND5	0.267
+7776C>T(0.162)	Cox2	0.25
+11035C>T(0.216)	ND4	0.25
+16051T>C(0.135)	D-loop	0.25
+16247C>T(0.162)	D-loop	0.25
+16255T>C(0.135)	D-loop	0.25
+12158T>C(0.189)	ND5	0.241
+15510C>T(0.189)	CYTb	0.241
+16093G>A(0.189)	D-loop	0.241

Three significant minor allele frequency were identified in 106, 222 and 16302 loci(Table 10). Meanwhile, the differences of MAF weren't distinct between HW and HS(Table 11). Minor allele frequency between HS vs JB showed significant difference in 106, 222, 16302, 12158, 15510 and 16093 loci(Table 12).

Table 11. Difference of minor allele frequency between HW and HS more than 0.2

SNPID2	Gene	HW-JB
+16247C>T (0.162)	D-loop	0.250



Table 12. Difference of minor allele frequency between HS and JB more than 0.2

SNPID2	Gene	HS-JB
+106T>D,C (0.270)	D-loop	1
+222C>D (0.216)	D-loop	0.857
+16302G>A (0.189)	D-loop	0.571
+12158T>C (0.189)	ND5	0.429
+15510C>T (0.189)	CYTB	0.429
+16093G>A (0.189)	D-loop	0.429
+2232G>A (0.054)	16s ribosomal RNA	0.286
+12908C>T (0.056)	ND5	0.286
+16042T>C (0.162)	D-loop	0.286
+16055T>C (0.054)	D-loop	0.286
+7776C>T (0.162)	COX2	0.2
+11035C>T (0.216)	ND4	0.2
+12684T>C (0.222)	ND5	0.2

To show genetic relationships among other breeds, identified polymorphisms of complete mtDNA were used for analyzing pylogenetic tree. *Bos taurus* breeds showed distinct distance from *Bos indicus* breeds(Fig. 6). Also, another phylogenetic tree using representative sequence of each breed showed distinct difference between *Bos taurus* and *Bos indicus*(Fig. 7).



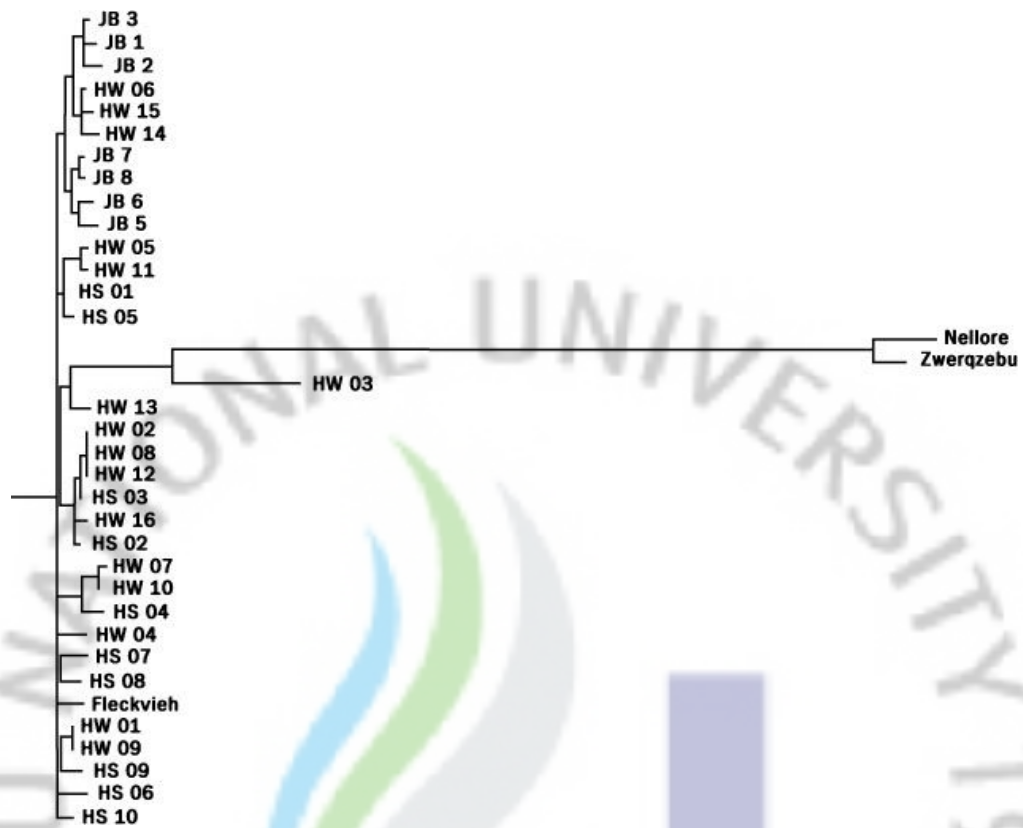


Fig. 6. Phylogenetic tree using the polymorphisms of 36 cattle.

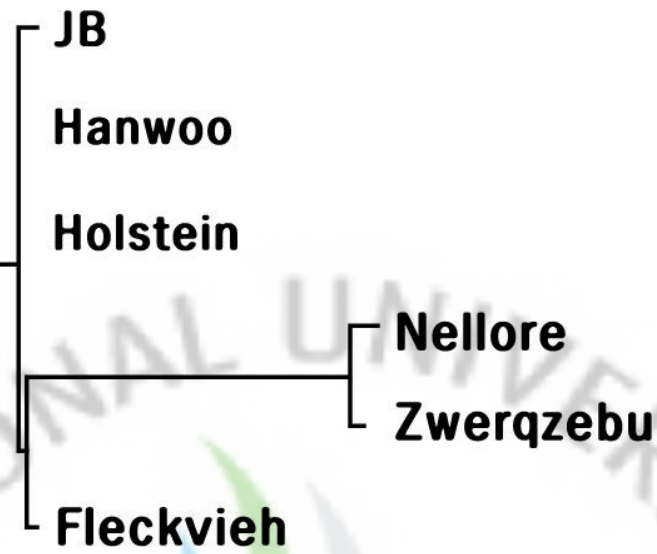


Fig. 7. Phylogenetic tree using representative sequence of each breed.

Also, *Bos taurus* breeds(Hanwoo, Japanese black cattle and Holstein) were separated from *Bos indicus* as Fig. 6.

Table 13. DNADIST among different breeds. *Bos taurus* breeds showed distinct genetic distance from *Bos indicus* breeds.

		<i>Bos taurus</i>			<i>Bos indicus</i>		
		HW	JB	HS	FLECK	NEL	ZWE
<i>Bos taurus</i>	HW						
	JB	0.0145					
	HS	0.0000	0.0145				
	FLECK	0.0169	0.0267	0.0169			
<i>Bos indicus</i>	NEL	0.8461	0.8843	0.8461	0.8631		
	ZWE	0.7976	0.8346	0.7976	0.8050	0.0692	

Genetic distance were calculated by DNADIST among breeds. If the value approaches "One", This means genetic distance becomes larger between two breeds. That the value approach "Zero" means genetic distance becomes closer than other comparative breeds. The value of DNADIST were shown from 0.000 to 0.267 within *Bos taurus*. Meanwhile, the value of DNADIST were shown from 0.7976 to 0.8631 between *Bos taurus* and *Bos indicus*. This means *Bos taurus* are separated from *Bos indicus* in the point of the genetic distance.

IV. Discussion

Each mitochondrion has its own genome, which copies for oneself. There are several hundred to several thousand mitochondria within a cell. This implies that the mitochondrial genome has a number of copies by amplification. Thus, mtDNA is the genetic system of choice even though tissue samples are very old, very small, or degraded by heat and humidity. One of representative variations is SNP(Single nucleotide polymorphism) including point mutations which weren't detected by restriction fragment length polymorphism(RFLP) as well as restriction endonuclease. Therefore, SNP are useful for genetic diagnosis, genetic marker for differentiating various breeds.

We have performed a direct sequencing of complete mtDNA including 16 HW and 10 HS and analyzed these sequences in conjunction with other sequence referred to NCBI. Through this analysis, 392 polymorphisms of 36 animals were identified including 4 breeds of *Bos taurus* and 2 breeds of *Bos indicus*. Because of comparing with various breeds, it was possible to present many polymorphisms in this study.

Though identified polymorphisms were distributed all over gene region, many polymorphisms existed in D-loop known as non-coding regions(76/392). As D-loop is non-coding region, hypervariable changes between nucleic acid happen more often than in other regions. Therefore, polymorphisms in D-Loop have been used for analyzing evolution of breeds and genetic difference of other breeds(Mannen *et al*, 1998; Kim *et al*, 2003; S. Tsuji *et al*, 2004).

Point mutations were divided into transitions and transversions. Transitions mean changes happen between the purines A and G, or between the pyrimidines C and T. and transversions mean changes happen between purines and pyrimidine. In

mammalian nuclear DNA, transitions happen to be almost twice as frequent as transversions(Belle *et al*, 2005). In this study, the most types(90.5 %) of nucleic acid substitutions were the result of transitions including A/G and C/T even though the exact mechanisms weren't clear. In addition, 16 Ins/Del type of 392 polymorphisms were identified including 2 trialleles, 3 heteroplasmies. In case 3 heteroplasmies, nothing exists in *Bos indicus*(Nellore, Zwergzebu) in this study.

When being compared with minor allele frequency between two breeds, low difference of minor allele frequency were identified between HW and HS.

In comparison of the minor allele between HW and JB, three polymorphisms(106, 222 and 16302 loci) showed significant difference of minor allele frequency(0.938, 0.857 and 0.384, respectively).

In comparison of the minor allele between HW and HS, one polymorphism(16247 locus) showed significant difference of minor allele frequency(0.250).

In comparison of the minor allele between HS and JB, 3 polymorphisms(106, 222, 16302 loci) showed significant difference of minor allele frequency(1, 0.85 and 0.571, respectively). On the basis of the results above, the study of sequence variation in bovine mitochondrial DNA gene can be useful for economic program regarding milk production traits between other breeds(Thuji *et al*, 2004).

As mentioned above, polymorphisms showing significant difference of minor allele frequency(>0.2) will be used as DNA markers for discriminating breeds.

In case of Fleckvieh, Nellore and Zwergzebu, they were excluded from the comparison between two breeds because of too small numbers of animals in this study.

To analyze genetic relationships among breeds, phylogenetic tree has been presented. Mitochondrial DNA sequences encoding proteins have been used for showing the phylogenetic relationships in various species(Irwin *et al*, 1991; Horai *et*

al, 1992; Adachi *et al*, 1993; Janke *et al*, 1994; Cao *et al*, 1994). For the phylogenetic tree analysis, complete mtDNA sequence were used in this study, too.

Using microsatellite polymorphisms for researching of genetic difference had been performed(MacHugh, 1997) in advance. The study affirmed the large scale of diversity between the *Bos taurus* and *Bos indicus* lineage previously reported for mitochondrial data(Manwell and Baker, 1980; Loftus *et al*, 1994a, b; Bradley *et al*, 1996). Also, the breeds of *Bos taurus*(Hanwoo, Japanese black cattle, Holstein and Fleckvieh) showed distinct differences from *Bos indicus*(Nellore, Zwergzebu) through analyzing phylogenetic tree in this study.

The 13th Hanwoo showed distinct difference from other Hanwoo in Fig. 6. This means Hanwoo have more various polymorphism than other breeds.

To identify the origin and genetic diversity of cattle in North Eastern Asia, mitochondrial analyses were previously performed in Japanese, Mongolian, and Korean native cattle. In that study, the results were that 20% of Mongolian cattle contained *Bos indicus* mitochondrial haplotypes, while, Japanese and Korean cattle contained only *Bos taurus* haplotypes(Mannen *et al*, 2004). Also, the study suggested the diversity within *Bos taurus* mtDNA types gave additional influence to the Mongolian cattle. The Korean and Japanese samples were composed of haplogroups which were also found throughout Europe, the Near East and especially East Asia. However, the origin of Hanwoo hasn't been demonstrated up to the present. Therefore, the research for the origin of Hanwoo will be needed through the analysis of various polymorphism.

V. Conclusions

We performed the direct sequencing of complete mtDNA in 26 cattle, which were Hanwoo and Holstein. To analyze polymorphism, sequences produced in this study were compared with 10 other sequences obtained from public database(NCBI). Also, to present genetic relationships among breeds, phylogenetic trees were shown. The conclusions are as follows:

1. The 392 mtDNA polymorphisms, including 376 SNPs, 13 Ins/Del and 3 heteroplasmies were identified.
2. Out of 392, 76(19%) polymorphisms were identified in hypervariable non-coding D-loop(76 sites/0.4 kb), which is much higher than other regions(316 bites /15.6kb).
3. Most of polymorphisms(355/392, 90.5%) identified in mtDNA were results of transition changes(A/G or T/C).
4. There has been low difference of minor allele frequency between Hanwoo and Holstein.
5. Phylogenetic analyses using mtDNA polymorphisms revealed distinct difference between *Bos taurus* and *Bos indicus*.

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국문초록

소 mtDNA의 유전적 다형성과 계통도에 관한 분석

김은희

(지도교수 : 윤영민)

제주대학교 대학원 수의학과

소의 전체 미토콘드리아의 염기서열이 밝혀진 이후로 다양한 품종의 미토콘드리아 DNA(mtDNA)를 확인하기 위한 많은 연구들이 진행되어왔다. 본 연구에서는 한우에서의 mtDNA의 유전적 다형성을 찾아내고 다른 품종들과의 유전적 관계를 분석하였다.

한우 16두와 홀스타인 10두에서 DNA를 추출하여 전체 mtDNA 염기서열을 분석하여 다른 품종의 염기서열과 비교 분석한 결과, 모든 품종에서 376개의 SNPs, 13개의 Ins/Del 다형성, 그리고 3개의 heteroplasmies를 포함하여 총 392개의 다형성이 확인되었다. 392개중 19%에 해당하는 76개의 다형성이 non-coding 되는 과변위 부분 D-Loop에서 확인되었다. mtDNA에서 대부분의 다형성 (355/392, 90.5%)은 transition에 의한 것들이었다(A/G or T/C). mtDNA 염기서열의 다형성을 바탕으로 한 계통도 분석에서 *Bos taurus*(한우, 일본소, 홀스타인, Fleckvieh) 품종과 *Bos indicus*(Nellore, Zwergzebu) 품종간에 명확한 유전적인 차이가 있음을 확인하였다.

본 연구에서 확인된 mtDNA의 유전적 정보는 향후 mtDNA를 이용한 다른 기초 및 응용 연구에 기초자료가 될 것이라 사료된다.

주요어: 미토콘드리아 DNA, 다형성, 계통도 분석, 한우, 홀스타인

감사의 글

논문 작업을 시작했을 때는 저의 부족함으로 인한 막연함 때문에 걱정스런 마음을 갖지 않을 수 없었습니다. 그러나 많은 분들의 도움과 격려 덕분에 이렇게 마무리를 하게 되어 기쁘고 감사합니다. 미약하나마 여기에 그 감사의 마음을 담아보고자 합니다.

논문 완성을 위해 많은 도움과 격려를 아끼지 않으시고 힘이 되어 주신 윤영민 교수님께 진심으로 감사드립니다. 물심양면으로 격려해주신 신형두 교수님께 감사드립니다. 늘 따뜻한 웃음으로 격려의 말씀 잊지 않으셨던 이경갑 교수님께 감사함을 전합니다. 저의 부족함을 같이 고민해 주고 도와주신 정현섭 선생님을 비롯한 SNP 선생님들께도 감사드립니다.

항상 좋은 말씀과 위로를 해주셨던 김재훈 교수님, 만날 때 마다 덕담 잊지 않으셨던 신태균 교수님, 넉넉한 미소로 반겨주신 정종태 교수님, 박현정 교수님, 많은 배려 해주신 이영재 교수님, 지영혼 교수님, 임윤규 교수님, 주홍구 교수님, 이두식 교수님, 이주명 교수님, 배종희 교수님, 황규계 교수님, 손원근 교수님, 강태영 교수님, 박전홍 교수님, 우호춘 교수님께 감사한 마음 전합니다.

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