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Identification of Genetic Markers for Korean Native Cattle (Hanwoo) by RAPD Analysis

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Abstract In order to develop the specific genetic marker for Korean native cattle (Hanwoo), randomly amplified polymorphic DNA (RAPD) analysis of 6 different cattle breeds was attempted by using 38 decamer primers. In comparison of RAPD patterns, two distinctive DNA bands specific for Hanwoo were detected. One was 296 bp of DNA fragment found to be specific only for female Hanwoo when primer GTCCACACGG was employed. In individual analysis of this RAPD marker was observed only in female individuals with the possibility of 85.3%. The other was 521 bp of RAPD marker amplified using TCGGCGATAG and AGCCAGCGAA primers, which showed 83.0% of genetic frequency in 85 male and 68 female individuals tested. Nucleotide sequencing of these genetic markers revealed that 296 bp marker has a short microsatellite-like sequence, ACCACCACAC, and a tandem repeat sequence of microsatellite GAAAAATG in the determined sequence. Two distinctive tandem repeats of microsatellite sequences, AAC and GAAGA, were also appeared in 521 bp DNA marker. In BLAST search, any gene having high homology with these markers was not found

Keywords: RAPD marker, Korean native cattle, Hanwoo, DNA polymorphism, genetic analysis

INTRODUCTION

Analysis of genetic polymorphism in higher organisms has been widely employed in estimating the genetic relatedness and diversity within and among species. Now this molecular approach became a powerful and reproducible tool in genetic analysis, breeding and identification of species and/or populations.

The most popular method for genetic variation analysis is DNA fingerprinting (DFP) by restriction fragment length polymorphism (RFLP). By this DFP by RFLP analysis, the DNA polymorphism is detected which can hybridize with multiple tandem repetitive sequences or hypervariable minisatellite [1,2]. Even though this method has been widely employed in genetic analysis of a variety of organisms, it has suffered from the requirements of large quantity of sample DNA and radioisotope handling.

Another approach for genomic polymorphism is randomly amplified polymorphic DNA (RAPD) analysis through DNA amplification by polymerase chain reaction (PCR) using primers of arbitrary sequences [3]. It has some advantages compared to DFP by RFLP; the simplicity and the rapidity as well as no requirement of the radioisotopes even though small amount of sample DNA is employed.

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In the case of cattle, lots of researchers have been made to detect DNA polymorphisms between cattle breeds by RAPD analysis during last decade [4-13]. Especially genetic markers for native cattles in each countries were strongly investigated for the improvement of their native cattle breeds, including Japanese Black cattle (Wagyu) [4], Germany native cattle [5], Zebu cattle [6], *Bos indicus,* and *Bos taurus* [7]. Several Korean researchers have also tried to find the genetic markers for Korean native cattles (Hanwoo) [9].

In this study, identification of new genetic markers more specific in Korean native cattle (Hanwoo) by analyzing the DNA polymorphisms by RAPD method, which are not detected in the other imported breeds in Korea. The identified genetic markers will be applied in the identification of Hanwoo and further the evaluation of their specific genetic traits.

MATERIALS AND METHODS

Preparation of Genomic DNA

The blood samples of Hanwoo were kindly supplied from Korean Federation of Animal Improvement (Seoul, Korea), and those of Holstein were taken in Animal Farm of Yeungnam University (Kyongsan, Korea). White blood cells collected from 10 mL of blood samples were lysed by SDS and proteinase K, and the genomic DNAs were extracted by phenol-chloroform method [13]. On

the other hand, the genomic DNA samples from 15 heads of Angus, Brahman, Charolais and Simmental were kindly provided by Dr. Young Hoon Yang in Cheju National University (Cheju, Korea)

Arbitrary Primers

The 38 oligonucleotide primers used in RAPD analysis (Table 1) were commercially supplied from Bioneer Co. (Cheongwon, Korea).

RAPD Analysis

25 μL of reaction mixture containing 0.5 unit of *Taq* DNA polymerase, 1 × *Taq* polymerase buffer (50 mM KCl, 10 mM Tris, pH 9.0), 2 mM $MgCl_2$, 200 μM of each dNTPs, 4 μM of primers and 100 ng/μL of genomic DNA, was subjected to 50 cycles of DNA amplification; 94°C for 1.5 min to denature, 36°C for 2 min for annealing of primer and 74°C for 2 min for extension using GeneAmp PCR System 2400 (Perkin-Elmer, CT, U.S.A.) [14]. The amplified DNA samples were separated on 1.4% agarose gels and visualized by staining with ethidium bromide.

DNA Cloning and Sequencing

The DNA band appeared on agarose gel was extracted using JET sorb kit (GENOMED Inc., NC, U.S.A.), and amplified again by PCR reaction with the same primer. The amplified PCR product was further purified with JET pure kit (GENOMED Inc., NC, U.S.A.). The isolated DNA fragment was ligated with pGEM-T vector (Promega Corporation, CA, U.S.A.) by T4 DNA ligase, and the ligated mixture was transformed into *E. coli* JM109 [15]. From the white colonies on LB (Luria-Bertani) media (1% tryptone, 0.5% yeast extract, 1% NaCl, pH 7.2) containing 50 μg/mL ampicillin, 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG), 0.04% 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal), the plasmid was isolated and confirmed to have insert DNA by carrying out PCR with the same primer. The nucleotide sequence of insert DNA was determined using universal M13/pUC sequencing primers by custom service of Bioneer Co. (Cheongwon, Korea). The determined sequence was further compared by BLAST search program (http://www.ncbi.nlm.nib.gov/blast).

RESULTS AND DISCUSSION

DNA Polymorphism of Various Cattle Breeds by RAPD Analysis

In RAPD analysis, primers with ten nucleotides and a (G+C) content of at least 50% are generally employed, because primers having high (A+T) content may cause DNA-primer hybrid melted during polymerization at 72°C [14]. In this study, 38 different decamer primers have been employed for the analysis of DNA polymorphism of Hanwoo and other cattle breeds (Angus, Brahman, Charolais, Holstein, and Simmental) by RAPD procedure (Table 1). Those primers were subjected as a

Table 1. RAPD primers used in this study

Primer No.	Sequence (5'→ 3')	Primer No.	Sequence (5'→ 3')
1	GTTGCGATCC	H8	GCCGCTACTA
2	CAAACGTCGG	H9	GGGCCCGAGG
3	GGTCCCTGAC	H10	TACGATGACG
4	AGTCAGCCAC	H11	TTCCCGGAGC
5	GACCAGCGAA	P1	CAGGCCCTTC
6	TTCCGAACCC	P2	CAATCGCCGT
7	CAGCACCCAC	P3	AGGGGTCTTG
8	TGCCAGGCTG	P4	TCGGCGATAG
9	TACCTAAGCG	P5	GAAACGTAGG
10	GTCCACACGG	P6	AGCCAGCGAA
11	GATCATAGCG	P7	GTGACGTAGG
12	CCCGCCGTTG	P8	TCCGCTCTGG
H1	AACGCGTAGA	P9	GGGTAACGCC
H2	ACATCCTGCG	P10	CTGAGACGGA
H3	AGCGGCTAGG	P11	GTGATCGCAG
H4	ATACGGCGTC	P12	TAGAACGAGG
H5	ATCGGGTCCG	P13	GTTTCGCTCC
H6	ATCTGCGAGC	P14	TGGATTGGTC
H7	CGGTGGCGAA	P15	GGACTGGAGT

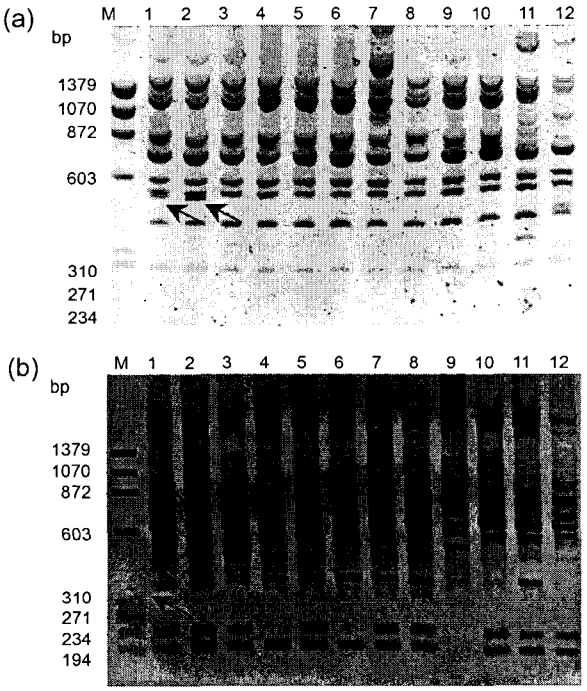

Fig. 1. DNA band patterns of cattle breeds detected by RAPD analysis. Primers of #P4 (TCGGCGATAG) and #P6 (AGCCAGCGAA) in (a), and #10 primer (GTCCACACGG) in (b) were employed in RAPD analysis. In each gels, lane M; Molecular marker (φX174 DNA/*Hae*III), lane 1, 2; Korean native cattle (Hanwoo), lane 3, 4; Holstein, lane 5, 6; Angus, lane 7, 8; Charolais, lane 9, 10; Simmental, and lane 11, 12; Brahman.

Table 2. Genetic frequency of genetic markers in individuals of Korean native cattle (Hanwoo) found by RAPD analysis

Primer	DNA Marker (bp)	Male			Female			Total		
		Tested	Positive	Frequency (%)	Tested	Positive	Frequency (%)	Tested	Positive	Frequency (%)
#10	300	85	0	0	68	58	85.3	153	58	37.9
#P4 +#P6	500	85	73	85.9	68	54	79.4	153	127	83.0

single primer or in the combination with other primers.

When the genomic DNAs isolated from white blood cells of 6 different cattle breeds were subjected as templates for RAPD analysis, two distinctive DNA bands specific for Hanwoo were found (Fig. 1). Especially when #10 primer (GTCCACACGG) was employed, 300 bp of DNA fragment was specifically amplified only in Hanwoo. The distinctive DNA band of 500 bp was also found only in the genomic DNA of Hanwoo when the combined primers of #P4 (TCGGCGATAG) and #P6 (AGCCAGCGAA) were employed.

However, Cho and Han [9] reported some specific DNA markers for Hanwoo, which was identified by RAPD analysis using #H4 (ATACGGCGTC), # H5 (ATCGGGTCCG), and #H9 (GGGCCCGAGG) primers, respectively. However, the same specific markers using the same primers were not found in this study. It might be due to the number of cattle breeds tested in RAPD analysis

It can be concluded that the genetic markers identified by RAPD analysis of DNA polymorphism in this study are more specific and more reproducible for Hanwoo. Furthermore, these results suggest that RAPD analysis procedure is a powerful tool for identification of genetic markers specific to Hanwoo, compared with other cattle breeds.

Genetic Frequency of RAPD Markers in Individuals of Korean Native Cattle (Hanwoo)

In order to confirm whether genetic markers found by RAPD analysis are the specific genetic markers only for Hanwoo, the genomic DNAs of 153 individuals consisting of 85 male and 68 female were subjected to RAPD amplification. As seen in Table 2, 85.9% of male Hanwoo and 79.4% female showed this specific DNA band in RAPD analysis using #P4 and #P6 primers.

In contrast, #10 primer gave the specific DNA bands of 300 bp only in female Hanwoo, but not in male. Probably, this band may be a unique DNA polymorphism found only in female Hanwoo.

Considering the history of Hanwoo breeding in Korea that Hanwoo had been cross-bred with other imported cattles during industrialization period in 1960s and the pure Hanwoo has not well kept in Korean urban, those are specific genetic markers uniquely present in Hanwoo, but not originally in other breeds.

DNA Sequences of RAPD Markers

These DNA markers for Hanwoo were cloned into pGEM-T vector of *E. coli,* and sequenced by dideoxy chain termination method. The determined nucleotide sequences of DNA markers are shown in Fig. 2 and Fig. 3.

GTCCACACGGGGGAAGGACCATGGTGTCCCAAG
TGGTGGGCAGGTTCATTCTTTTCTCTCAGAAAAG
GAGGACAAGGAGAGAAAAGCTTCTAGTTTAATGTC
CTGCCAGCAGAACAGACTGTAGAATGAGGCTGCT
ACATGCCAACGAGCGAGAGGAACCAGTGCGTTTG
TGGTGGGTATCCCAGGACTTTATAACCCCTGACA
CTACCACCACACGTGTGTTGCTTGAACCTGCTCA
TTGGAGGATGAAAAATGAAACGAAGAAAAATGTCC
TCGTAACTTAGTGCCGTGTGGAC

Fig. 2. The nucleotide sequence of 296 bp RAPD genetic marker specific only for female Korean native cattle (Hanwoo). The underlined thick letters at both ends shows the #10 primer (GTCCACACGG). The letters in gray boxes shows a tandem repeat sequence of microsatellite, and the underlined letters at the middle part shows a microsatellite-like sequence.

AGCCAGCGAAACCAGCCTTCCACAGCTAATCTCC
AGCACAGTAAAATAATACATTTGTATTTTTCGAAG
CTAAAAAAGGTAACAAAATATCATGCCCTTGGCGT
CACAGCTGAAAAGAACTTCCCAGATCTAGTACAAT
CCTGATGGAAATATGGTGAGAAAGATGGTCTTTTC
AAGGTCACATAGCTAGTGGTCCTATTTATTTTAAA
AAACAACAACAACAACGTTTCCTCCTCAAAATAAA
GAGATAGGAAGAGAAGAAGCAATGAATGGGGTTAT
TATTCTAATTTTATGAGAAGAAACAAATTCACTGAA
TTAAGTAACTTGGGCCATTACCTCATATCTAATAC
TGTGTTTTAAGTATTTAAAAACATCGAAGTAATGAA
GGTACATGAATGATAATTTTAAAAAATCACATCATA
GCAGACAGAAGGACTTATCATGAATAGCATTCATT
TCTGTGCTGTTCTTAAGTCTCTCAGTTGTGTCCG
ACTCTTTGCAACCCTATGGACTATCGCCGA

Fig. 3. The nucleotide sequence of 521 bp RAPD genetic marker specific for Korean native cattle (Hanwoo). The underlined thick letters at both ends shows the #P4 (TCGGCGATAG) and #P6 (AGCCAGCGAA) primers. The letters in gray boxes at the middle part shows two tandem repeats of microsatellite sequence.

The 296 bp genetic marker amplified with #10 primer, which is specific only for female Hanwoo, has a tandem repeat of microsatellite GAAAAATG in the sequence. In the middle part of sequenced DNA, a short microsatellite-like sequence, ACCACCACAC was also found.

On the other hand, two distinctive tandem repeat sequences of microsatellite, AAC and GAAGA were lo-

cated at the middle of 521 bp DNA marker found by RAPD analysis using #P4 and #P6 primers. However, any other tandem repeat sequence longer than 5 bases was not found in this marker.

Using the BLAST search program, these DNA markers specific for Hanwoo was compared with 482,057 known sequences. The searching results said that there is not any highly homologous gene in databases. The sequence showing the highest matching score with 521 bp genetic marker was the repeat region located between bovine glutamine tRNA gene and serine tRNA gene (E = 2e-09, 97% matching between base 476 and base 513) [16]. However, any gene showing significant matching score with 296 bp genetic marker was not found in the BLAST search.

It implies that the identified RAPD marker can be successfully used in differentiating DNA polymorphism of cattles, especially in evaluation and identification of Hanwoo. If this marker can be linked with quantitative trait loci for economically important traits, it would contribute in marker-assisted programs for Hanwoo breeding.

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