Detection of DNA Fragment to Differentiate Korean Cattle

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ABSTRACT : In order to identify and develop the specific DNA marker for the identification of Hanwoo (Korean Cattle) from other breeds, a specific DNA marker of 519 bp was identified and sequenced from polymorphic analysis using RAPD-PCR for 6 cattle breeds. Two different repetitive sequences, $(AAC)_5$ and $(GAAGA)_2$, were selected and designed to use specific probe to develop a DNA marker for Hanwoo specific. When the $(AAC)_5$ probe was applied, the 10 kb specific DNA marker showed in the DNA fingerprinting from 237 of 281 Hanwoo individuals. This novel Hanwoo specific DNA probe is useful to perform the marker-assisted selection for screening Hanwoo purity as an unique genetic source. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 8 : 1071-1075)***

Key Words : RAPD-PCR, Specific DNA Probe, DNA Fingerprinting, Hanwoo (Korean Cattle)

INTRODUCTION

Genetic erosion and extinction threaten an increasing number of plant and animal species. the major consequence being a loss of global genetic diversity requires sets of genetic markers that characterize distinct populations.

The Korean cattle breed, Hanwoo. is of particular importance in Korea because of its favorable meat quality although its productivity is relatively low in general. Unfortunately. this local breed has been threatened with genetic erosion through crossbreeding. Thus. it is important to identify genetic characteristics of this breed for establishing breeding parameters and conservation. Breed characterization requires knowledge of genetic variation that can be effectively measured within and between population (Hetzel and Drinkwater. 1992), for example, in terms of mitochondrial and nuclear DNA polymorphisms (Rassmann et al.. 1991; Suzuki et al.. 1993; Tautz. 1992). A recent technique capable of detecting DNA polymorphism is based on random amplification by the polymerase chain reaction (PCR) of DNA segments with short oligonucleotide primers. and annealing PCR (RAPD-PCR). has been successfully applied in genetic studies of various species (Michelmore et al., 1991; Welsh and McClleland. 1991), as well as for characterization of bovine populations (Bardin et al.. 1992; Kemp and Teale. 1992). Different primers produce different RAPD polymorphisms, the variations being due to differences in spacing between primer binding.

Like all other techniques. the RAPD-PCR has limitations, amongst which is the relative complexity of resultant fingerprint patterns and the fact that heterozygotes cannot be distinguished from homozygotes. However, the ease with which many RAPD polymorphic loci can be identified without primer sequence information. and the direct reading of results from agarose gels, make the RAPD techniques useful in large outbred populations. Here. we report results of applying the RAPD-PCR technique to the differentiation of Hanwoo cattle, which represent valuable animal genetic resources in Korea.

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MATERIALS AND METHODS

Experimental animals

Blood sample were obtain from a total of 281 head of Hanwoo. 15 head of each foreign breeds and two Asian breeds. The imported foreign breeds included Holstein. Charolais, Simmental, Aberdeen Angus and Brahman. Two Asian breeds were Japanese Black and Chinese Yanbian which have the same origin as Hanwoo.

Genomic DNA isolation and RAPD analysis

White blood cells were collected and lysed by SDS and proteinase-K. Genomic DNA was extracted by the phenol-chloroform method. Sixty different 10-base oligonucleotide primers for RAPD-PCR analysis were supplied by custom service of Bioneer Co. (Cheongwon. Korea). PCR amplifications were performed in a 25 μl reaction containing 0.5 U Taq DNA Polymerase (Takara, Japan), 1X PCR buffer (50 mM KCl, 10 mM Tris-HCl. pH 8.3, 0.01% gelatin). 0.2 mM of each dNTP. 200 pmol primers and 50 ng of genomic DNA templates with the following temperature cycles: 5 min at 94°C followed by 50 cycles of 94°C for 1 min. 36°C for 2 min and 72°C for 2 min. Five microliters of PCR amplified DNA fragments were separated by electrophoresis onto 1.5% agarose gels.

DNA cloning and sequencing

The DNA was extracted from a gel slice using the JET sorb kit (GENOMED Inc., NC, USA) and amplified again by PCR reaction with the same primer. The amplified PCR product was further purified with JET pure kit (GENOMED Inc., NC, USA). This DNA fragment was cloned into pGEM-T vector (Promega Corporation, CA, USA) and transformed into E. coli JM109 (Sambrook et al., 1989). The nucleotide sequence of insert DNA was determined using universal sequencing primers by custom service of Bioneer Co. (Cheongwon, Korea).

Probe design and DNA fingerprinting

Repetitive sequences like microsatellite probes of 519 bp for identifying Hanwoo breed were designed from DNA sequence produced by RAPD. Two micrograms of synthesized single stranded oligos [$(AAC)_5$ or $(GAAGA)_2$] were ligated using T4 DNA ligase, and amplified by PCR.

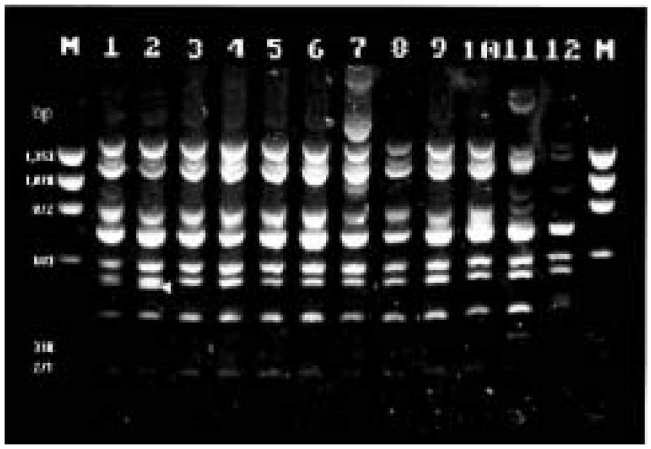
Fifty micrograms of genomic DNA digested with a restriction endonuclease, *Pst* I, was separated by electrophoresis in a 1.2% agarose gel with 40 cm length. Hybridization was carried out using ^{32}P-α-dCTP radioactive end-labeled probe for 15 h at 37°C in hybridization buffer (6X SSC, 40% formamide, 5 mM EDTA and 0.25% skim milk; Sambrook et al., 1989). Hybridization membranes were washed twice with 4X SSC, 0.1% SDS and once with 2X SSC, 0.1% SDS for 30 min at 55°C. The final washed membranes were exposed for 4 days to X-ray film (Fujii, Japan) for autoradiography.

RESULTS

DNA Polymorphism of various cattle breeds by RAPD-PCR

Sixty random primers were used to identify specific DNA markers to distinguish Hanwoo from five foreign cattle breeds. Ten individual's DNA isolated from each cattle breed were pooled and subjected to RAPD-PCR analysis with the combined primers of No. 4 (5'-TCGGCGATAG-3') and No. 6 (5'-AGCCAGCGAA-3'). We found that a DNA band of 519 bp from RAPD-PCR products was shown only in Hanwoo breed (figure 1). This specific band of 519 bp was a unique DNA marker for Hanwoo breed.

Even though RAPD marker may be informative and a useful means by itself, its use has been limited due to extreme sensitivity to amplification conditions and poor reproducibility. It is, therefore, necessary to develop new primers or DNA probes from RAPD markers. We cloned the DNA band of 519 bp, a unique genetic marker for Hanwoo, by the Polymerase Chain Reaction (figure 2). Interestingly, two repetitive sequences of $(AAC)_5$ and $(GAAGA)_2$ were found in the sequence.



Lane 1, 2: (Korean Cattle) Hanwoo, Lane 3, 4: Holstein
Lane 5, 6: Angus Lane 7, 8: Charolais
Lane 9, 10: Simmental Lane 11, 12: Brahman
M: OX 174/*Hae* III digest Marker

Figure 1. RAPD-PCR band patterns of cattle breeds which amplified with random primers.

Probe design and southern analysis

Under the assumption that this specific marker for Hanwoo shows different DNA polymorphisms compared to other breeds, primers and microsatellite probes were selected from the determined sequence. The selected DNA fragments, $(AAC)_5$ and $(GAAGA)_2$ were synthesized with complementarity. The designed DNA probes were confirmed as propriety in differentiating Hanwoo from the other cattle breeds. Southern blot analysis was performed on 15 individuals selected form each cattle breed.

As shown in figure 3, there was a distinctive marker of 10 kb, which presumably consisted of DNA with only repetitive sequence of $(AAC)_5$, in DNA fingerprinting of Hanwoo, whereas the 10 kb marker was not shown in foreign cattle breeds. However the same 10 kb genetic marker for Hanwoo was observed in two individuals of Chinese Yanbian breed, implicating that Hanwoo is more closely related to Chinese Yanbian than Japanese Black (Shin et al., 1999).

Hanwoo breed specificity of RAPD fingerprints

In order to confirm the reproducibility, the $(AAC)_5$ probe was applied to DNA fingerprinting of the Hanwoo population. A total of 281 head of Hanwoo were tested by DNA fingerprinting with $(AAC)_5$, including 109 individuals registered as phenotypic standards at the Hanwoo Genetic Improvement Center, 126 selected by the Korean Animal Improvement Association, 20 from local farms, and 26 assumed as doubtful ones by the above national agencies. As shown in figure 4, progeny-tested bulls from the Hanwoo Genetic Improvement Center and pedigreed individuals from the Korean Animal Improvement Association were neither clear phenotypes nor carrying the

5'- AGCCAGCGAA ACCAGCCTTC CACAGCTAAT CTCCAGCACA GTAAAATAAT
ACATTTGTAT TTTTCGAAGC TAAAAAAGGT AACAAAATAT CATGCCTTGG 50
CGTCACAGCT GAAAAGAACT TCCCAGATCT AGTACAATCC TGATGGAAAT 100
ATGGTGAGAA AGATGGTCTT TTCAAGGTCA CATAGCTAGT GGTCCTATTT 150
ATTTAAAA*AA CAACAACAAC AAC*GTTTCCT CCTCAAAATA AAGAGATAG*G* 200
*AAGAGAAGA*A GCAATGAATG GGGTTATTAT TCTAATTTTA TGAGAAGAAA 250
CAAATTCACT GAATTAAGTA ACTTGGGCCA TTACCTCATA TCTAATACTG 300
TGTTTTAAGT ATTTAAAAAC ATCGAAGTAA TGAAGGTACA TGAATGATAA 350
TTTTAAAAAA TCACATCATA GCAGACAGAA GGACTTATCA TGAATAGCAT 400
TCATTTCTGT GCTGTTCTTA AGTCTCTCAG TTGTGTCCGA CTCTTTGCAA 450
CCCTATGGAC TATCGCCGA -3' 500 519

Figure 2. DNA sequence and designed DNA fragments of specific marker from RAPD-PCR in Hanwoo cattle □ :primers. ___ :repetitive sequence.

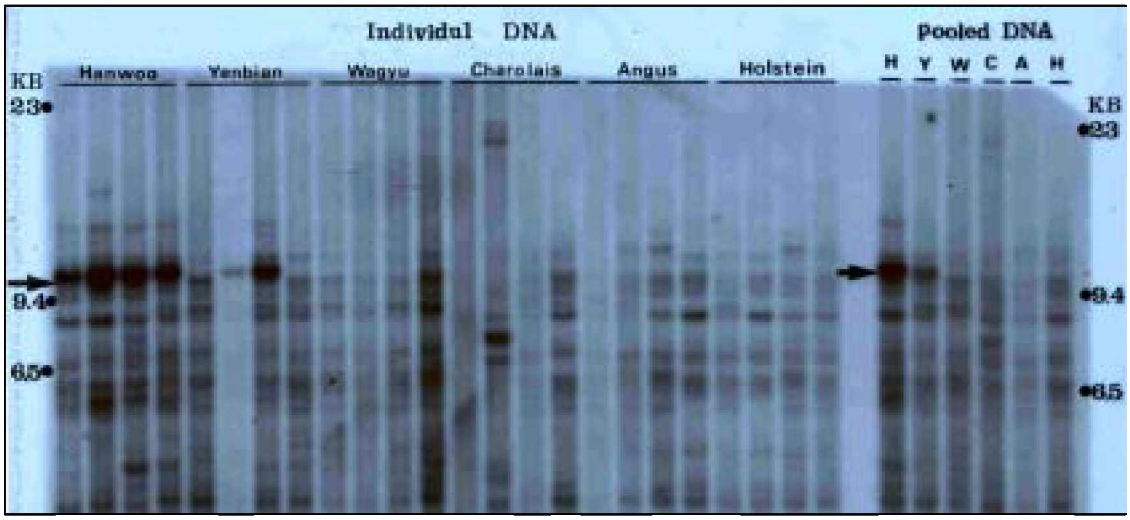


Figure 3. DNA fingerprinting of 6 breeds of cattle in individual DNA samples at right side and pooled sample of right side using designed probe $(ACC)_5$ and restriction enzyme *Pst*I (Arrows: specific marker of Hanwoo).

specific marker. Therefore, most of individuals from local farms were not proven to retain genetic purity (table 1).

DISCUSSION

This study demonstrated that the short random oligonucleotides of arbitrary sequences are useful to use DNA markers to distinguish individual animals, populations and cattle breeds. We have used this approach to successfully differentiate Hanwoo breed from foreign cattle by variation of RAPD fingerprints in breed-specific DNA pools and subsequent follow-up of prominent fingerprints in individual animals.

Kemp and Teale (1994) identified population-specific DNA polymorphisms to detect *Bos indicus* and *Bos Taurus* in West Africa. It was estimated that allele frequency is characteristic of cattle breeds using DNA markers (Buitcamp et al., 1991; Mannen and Tsuji, 1993; Glowatzki-Mullis et al., 1995; Choy et al., 2001).

Since it has been generally considered that Korea, Japan and China in Far East Asia share common historical and cultural backgrounds, it is assumed that their cattle breeds would also be of the same origin. Under this historic background, Japanese Black cattle has been improved with quite different aims and breeding system compared to Korean Hanwoo and Chinese Yanbian cattle which had relatively lower performance and genetic homogeneity. As indicators of genetic homogeneity, the heterozygosity of

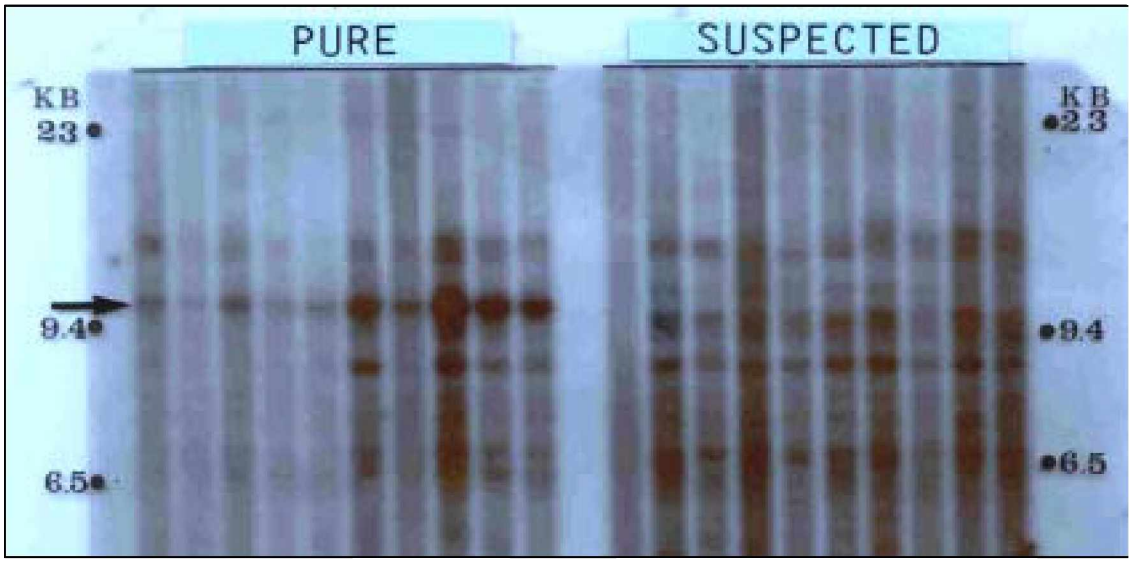


Figure 4. Differentiated DNA marker of Hanwoo standardized with registered phenotypes and doubted to cross with other breeds using designed probe $(AAC)_5$ and restriction enzyme *Pst*I (Arrows: specific markers of Hanwoo).

Table 1. Distribution of specific markers in Hanwoo (Korean Cattle) by $(ACC)_5$/PstI

Source	No. of head	Specific marker(10kb)	
		Yes	No
CGIC-NACF[1]	109	109	0
KAIA[2]	126	126	0
Local Hanwoo farm	20	2	18
Doubtful individual	26	0	26

[1] Cattle Genetic Improvement Center, National Agricultural Cooperatives Federation.
[2] Korean Animal Improvement Association.

Hanwoo and Yanbian were higher than Japanese Black. Inbreeding coefficients of Hanwoo and Yanbian were lower (Shin et al., 1999).

During a few decades of systematic breeding for Hanwoo, breeding efficiency of important characteristics such as growth and marbling have not been satisfactory. However genetic purity of basic population has been determined only by phenotypic characteristics. Expected genetic gain of performance would be based and variation of performance should be wide. When genetic uniformity of experimental materials is not established, it could be difficult to get uniform results from the feed-lot trial to produce high quality beef. Therefore, to make a genetic uniformity and distinguish the basic population of Hanwoo from the hybridized individuals genetically, we differentiated Hanwoo from other cattle breeds by using a DNA probe to determine one of genetic constitutions of Hanwoo. Thus, we suggest that performing DNA marker analysis is necessary for strategies for improvement of Hanwoo, preservation as systematic registration, identification of population for breeding, and distinguishing Hanwoo beef from others.

A similar situation has been reported for Tanzanian Zebu cattle breeds that could be screened 61-89% genetically from foreign cattle (Gawakisa et al., 1994). It might be due to the breeding history of Hanwoo during the Korean industrialization period in 1960's when Hanwoo breeds had not been well maintained by cross-breeding with foreign imported cattle in Korea.

Until now, polymorphic DNA analysis of Hanwoo and its application in breeding have been considered to be insignificant. However, the necessity for genetic improvement using DNA markers is now strongly emphasized in accordance with the current research trend. In this study, development of a new DNA fragment as a probe for DNA fingerprinting was attempted using DNA samples from individuals of different cattle breeds. This probe could be used in screening of population-specific markers for the detection of pure and cross-breeds, and further used in accurate basic-population analysis for genetic research of Hanwoo.

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