

Application of RAPD Methods in Meat for Beef Breed Identification

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ABSTRACT : Bovine genome samples were collected from meat of three different beef breeds (Hanwoo, Holstein and imported beef breed) that are commercially merchandized in Korean beef market. Operon B (OPB)-kits were used as random primers (3, 7, 10, 11, 12, 14) in random amplified polymorphic DNA (RAPD) method on whole genome. Each primer provided characteristic bands that were highly polymorphic. Each single primer could provide relatively efficient polymorphic band patterns among breeds. However, use of two or more primers in combination is recommended to improve resolution of experiments with higher molecular weight bands of DNA. In our experiments, OPB-11 resolved well between beef cattle breeds and Holstein. And OPB-7, 12 and 14 could be combined with OPB-11 to identify Hanwoo beef from the other two kinds of beef. (*Asian-Aust. J. Anim. Sci.* 2001. Vol 14, No. 12 : 1655-1658)

Key Words : Hanwoo, Holstein, Random Amplified Polymorphic DNA

INTRODUCTION

Korean beef market flow would be greatly challenged by initiation of free international trade from the year 2001. Another problem present in beef market is unstable market flow of beef from producers to consumers. Brand marketing of domestic beef producers are in tragedy by differences in grading system from country to country and by misidentification of imported beef as domestic Hanwoo beef which is recognized as the best quality and the most expensive beef in domestic market. Therefore, there is a great need for a new, clear and rather cheap technology to identify the sources of beef products. Several approaches for identification were tried, for example, physical structure analyses with the aids of eye or optical and electron microscopy (Kang et al., 1992; Oh, 1996), chemical analyses by chromatography or by electrophoresis of amino acids or proteins (Ashoor et al., 1988; Dincer et al., 1987; Hoyen and Thorson, 1970; King and Kurth, 1982; Yman and Sangberg, 1987), and immunoassay with electrophoresis or with ELISA (Allsup, 1987; Casas et al., 1985; Hayden, 1981; Hitchcock and Crimes, 1985; Kangethe and Gathuma, 1987; Patterson and Sencer, 1985). However, the resolution of these methods is greatly affected by environmental variation such as feeding, disease and physical environments.

Recent developments in molecular biology let us to find out species specific DNA markers, which are independent of environmental variation (Chikuni et al., 1990). The frequently used method in meat science, restriction fragment length polymorphism (RFLP) is expensive and requires use of radioisotope (Han et al., 1993). Instead, random amplified polymorphic DNA (RAPD) technique is simpler and more economic than RFLP (Lee et al., 1994;

Lee and Sang, 1995; Min et al., 1995).

The objective of this study is to identify DNA polymorphisms among different sources of beef using RAPD technique.

MATERIALS AND METHODS

DNA extraction

Genomic DNA was isolated by the method of Blin and Stafford (1976). One grams of tissue were sliced from the samples and directly frozen with liquid nitrogen. After grinding, tissue samples were mixed with 12 ml of digestion buffer (0.1 M NaCl, 0.01 M Tris HCl pH 8, 0.025 M EDTA pH 8, 0.5% SDS, 0.1 mg/ml proteinase K) and digested in a 50°C water bath for 18 h. After digestion, the samples were mixed gently with the same amount of phenol-chloroform-isoamyl alcohol (25:24:1) for 3 minutes to make sure that the whole mixture became homogenous. The mixture was then centrifuged at 1,700×G for 10 minutes. Supernatant of the mixture was transferred to a new container with wide mouth pipette and mixed with a mixture of 0.75 volumes of 5 M ammonium acetate (NH₄OAc) and 2 volumes of pure (100%) ethyl alcohol.

The pellets obtained following another centrifugation at 12,000 rpm for 30 minutes were washed with 70% alcohol. Washing pellets with 70% alcohol were repeated twice again after centrifugation for 10 minutes in between. Ethyl alcohol residue was removed from pellets by aspirator. DNA pellets were then dried in vacuum desiccators and stored in 0.1 ml TE (Tris EDTA) solution.

Amplification of DNA using PCR

A mixture of 50 ng DNA, 100 mM Tris-HCl (pH 8.3), 400 mM KCl, 15 mM MgCl₂, 0.5 ng/ml BSA (Bovine, 2.5 µl reaction buffer containing 10 mM DTT (Dithiothreitol), 5 pM primer, 200 mM dNTP (deoxy N-triphosphate; N=A, T, C, G), 0.125 unit Taq polymerase and

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sterilized water was put in a 0.5 ml PCR tube (Perkin Elmer Inc., Wellesley, MA, USA). After adding 22 μ l mineral oil into the tube, DNAs were amplified by 45 cycles, each of which was run at 95°C for one minute, at 39°C for one minute and at 75°C for two minutes.

Amplified DNA samples were applied to electrophoresis of 1% agarose gel. The gel dyed with EtBr (Ethidium Bromide) was washed with distilled water and photographic image was taken with a Polaroid camera (SL-5GD photographic system, Polaroid Co. Cambridge, MA, USA). The size of PCR products in base pair (bp) units were calculated using semi log table.

We used OPB-3, 7, 10, 11, 12, 14 among Operon B kits (Operon Tech. Inc., Alameda, CA, USA) as primers in our experiments. The DNA base sequences of the primers are listed in table 1.

RESULTS AND DISCUSSIONS

Figure 1 shows band patterns of Hanwoo, domestic Holstein and imported beef DNAs amplified by OPB-07 as primer. All three samples showed bands at 1,100 and 600 bps. Domestic Holstein beef sample (lane 2) showed a unique band at 1,200 bps.

Figure 2 is the band pattern of three beef samples by OPB-12 (lanes 1 through 3) and OPB-14 (lanes 4 through 6). Band at 1,350 bps by OPB-12 was only found with domestic Holstein beef (lane 2) while bands at 1,100 bps were observed only with Hanwoo and imported beef samples. We could observe bands at 900 and 790 bps in all three samples. We could also observe bands at 840 bps in domestic Holstein beef and imported beef samples except in Hanwoo beef sample. With OPB-14, bands at 1,650, 1,400, 1,250 and 1,150 bps were observed with Hanwoo beef sample. Bands at 1,050 bps were found only in domestic Holstein and imported beef samples. All three samples showed bands at 840 bps while a unique band for imported beef sample was found at 720 bps. Common bands were also observed at 630 bps for all three samples. Light bands for Hanwoo or imported beef samples were also found at 580 bps.

Band patterns out of OPB-3 (lanes 1~3), OPB-10 (lanes 4~6) and OPB-11 (lanes 7~9) are presented in figure 3.

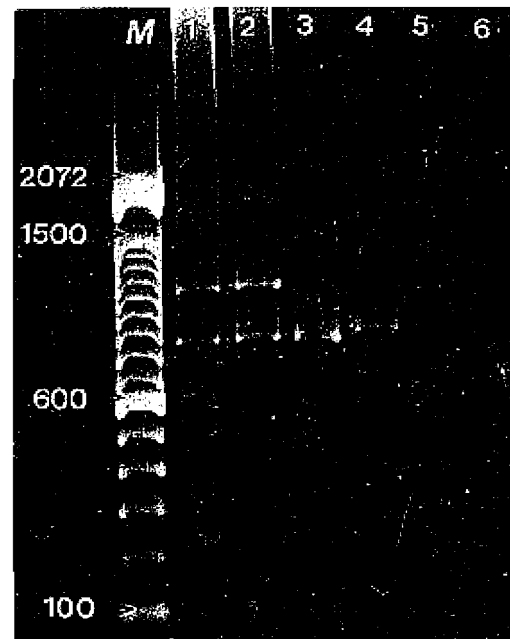
Table 1. Base sequences of random primers of Operon B-kit

Primer	Sequences
OPB-03	5' - CAT CCC CCT B - 3'
OPB-07	5' - GGT GAC GCA G - 3'
OPB-10	5' - CTG CTG GGA C - 3'
OPB-11	5' - GTA GAC CCG T - 3'
OPB-12	5' - CCT TGA CGC A - 3'
OPB-14	5' - TCC GCT CTG G - 3'



Lane 1: Hanwoo
Lane 2: Korean Holstein
Lane 3: Imported beef breed

Figure 1. RAPD patterns of beef breeds using OPB-07 as primer



Lane 1 and 4: Hanwoo
Lane 2 and 5: Korean Holstein
Lane 3 and 6: Imported beef breed

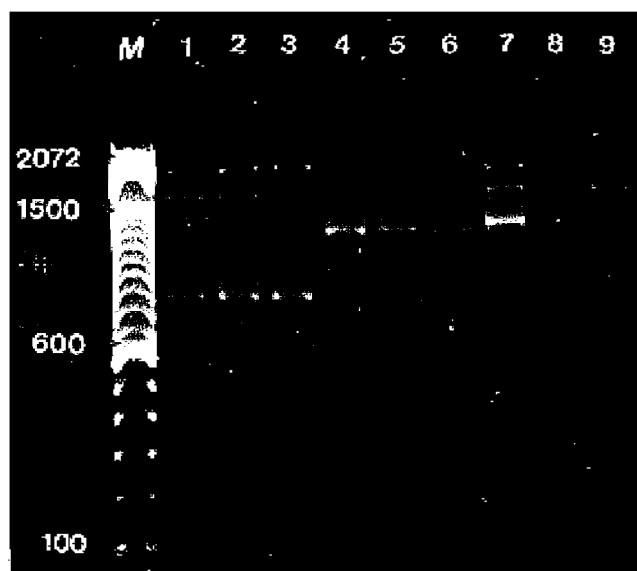
Figure 2. RAPD patterns of beef breeds using OPB-12 (lanes 1 through 3) and OPB-14 (lanes 4 through 6) as primer

Using primer OPB-3, characteristic bands were found at 780, 1,600 and 1,800 bps. Bands at 1,300 and 1,100 bps were only observed in imported beef samples. Bands at 1,200 bps were, on the other hand, observed only in

Table 2. Summary of band patterns* by primers excluding bands which appear commonly in all three breeds

Primers	bp,	1,800	1,600	1,500	1,300	1,200	1,100	900	800	700	600	500
OPB-03	Hanwoo											
	Holstein					✓1,200						
	Imported Beef				✓1,300		✓1,100					
OPB-07	Hanwoo											
	Holstein					✓1,200						
	Imported Beef											
OPB-10	Hanwoo						✓1,100					
	Holstein											
	Imported Beef											
OPB-11	Hanwoo	✓1,800	✓1,600	✓1,500	✓1,300		✓1,100	✓940	✓850		✓650	✓550
	Holstein											
	Imported Beef	✓1,800	✓1,600		✓1,300							
OPB-12	Hanwoo						✓1,100					
	Holstein				✓1,350				✓840			
	Imported Beef						✓1,100		✓840			
OPB-14	Hanwoo		✓1,650		✓1,400	✓1,250	✓1,150					✓580
	Holstein							✓1,050				
	Imported Beef							✓1,050		✓720	✓580	

* Numbers after ✓ represents molecular weight of the bands at reference marker; for example, ✓1350 means that the band was found at the reference region of 1,350 bp.



Lane 1, 4 and 7: Hanwoo
 Lane 2, 5 and 8: Korean Holstein
 Lane 3, 6 and 9: Imported beef breed

Figure 3. RAPD patterns of beef breeds using OPB-03 (lanes 1 through 3), OPB-10 (lanes 4 through 6) and OPB-11 (lanes 7 through 9) as primer

domestic Holstein beefs. With OPB-10, Hanwoo samples showed characteristic bands at 1,100 bps with some brighter bands at 1,230 bps compared with bands of the other beef samples commonly shown at 500, 940, 1,230 and 1,880 bps. DNA Bands amplified with OPB-11 showed no observable bands with domestic Holstein beef samples. However,

bands at 1,300, 1,600 and 1,800 bps were observed with Hanwoo and imported beef samples. And bands characteristic solely to Hanwoo were found at 550, 650, 850, 940, 1,100 and 1,500 bps.

Band patterns found in figures 1 through 3 are summarized in table 2. This table shows clearly that any single primer can be used to identify breed origin of beef. Considering low resolution of bands from light molecular weight, combination of primers can be recommended to clarify polymorphism. Desirable combinations found in our experiment may be application of OPB-11 with OPB-14, OPB-12 or OPB-7 because OPB-11 can be used to identify beef cattle breeds and Holstein breed and the others can identify Hanwoo or imported beef breeds from each other. These primers supply relatively better resolution of DNA bands in higher range of molecular weight, over 1,100 base pairs.

To summarize, banding patterns were different depending on the primers used. And RAPD method gave efficient tool for identification among beef of several different genetic origins. Several bands specific to each primer can be applied to distinguish Hanwoo beef from domestic Holstein beef or imported beefs. However, the difference in gene flow between imported and domestic *Bos indicus* cattle should be reconsidered through more detailed family structure analyses and mating design.

IMPLICATIONS

Based on the results of this study, we suggest that

polymorphic markers out of RAPD method can be used for identification of beef from different breeds. However, more detailed experiments that can deduce phylogenetic configuration should follow to prove the genetic differentiation on those markers.

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