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Utilization of DNA Marker-Assisted Selection in Korean Native Animals

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Abstract The recent progress of DNA technologies including DNA fingerprinting (DFP) and random amplified DNA polymorphism (RAPD) analysis make it possible to identify the specific genetic traits of animals and to analyze the genetic diversity and relatedness between or within species or populations. Using those techniques, some efforts to identify and develop the specific DNA markers based on DNA polymorphism, which are related with economic traits for Korean native animals, Hanwoo (Korean native cattle), Korean native pig and Korean native chicken, have been made in Korea for recent a few years. The developed specific DNA markers successfully characterize the Korean native animals as the unique Korean genetic sources, distinctively from other imported breeds. Some of these DNA markers have been related to some important economic traits for domestic animals, for example, growth rate and marbling for Hanwoo, growth rate and back fat thickness for native pig, and growth rate, egg weight and egg productivity for native chicken. This means that those markers can be used in important marker-assisted selection (MAS) of Korean native domestic animals and further contribute to genetically improve and breed them.

Keywords: Korean native animal, DNA marker, economic traits, marker-assisted selection

INTRODUCTION

Korean native animals such as cattle (Hanwoo), pig and chicken, had been preserved for several thousand years as the unique genetic sources in Korean Peninsula. Especially, Hanwoo has been sustained as symbol of wealth in traditional Korean life since they were employed in agricultural cultivation for a long time until their beef production became favorable to Korean consumers in 1970s. On the other hands, Korean native pig and chicken have been nearly diminished after early of 20th century because of their low productivity compared to foreign imported breeds and our indifference to keeping them under Japanese colonization. Therefore, Korean native pig and chicken as Korean unique genetic sources were almost extinct through the hybridization with foreign breeds.

Nowadays, the necessity to sustain and preserve Korean native animals has been gradually emphasized by consumers' higher requirement of meat quality of native animals with the increment of economic level, and by realization of importance of Korean unique genetic resources in the open era of meat market under World Trade Organization (WTO) treaty. Especially, superior meat quality of Korean native animals based on the satisfaction with Korean-preferred flavor makes now Korean native meat compete with imported one. With deep considerations on Korean native genetic sources, several researchers have put their efforts to improve the performances of Korean native animals for last a few decades. However, limitation of genetic capacities of native animals brought some difficulties in animal improvement for increasing their productivity. In accordance with scientific progress, new breeding strategy on molecular basis offers new way to identify distinctions of Korean native animals from other foreign animal breeds beyond traditional methodology. It will give answers for "What is the genetic characteristics of Korean native genetic sources?" and "How will they be further improved?"

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MOLECULAR APPROACH FOR THE ANALYSIS OF DNA POLYMORPHISM

In modern genetics, the development of molecular markers based on the polymorphism of organisms has been a greatly important research area in a variety of disciplines such as taxonomy, phylogenic analysis, genetics and breeding. Early days molecular markers based on protein polymorphism had been generated through allozyme analysis from tissue extracts [1]. However this method did not provide so sufficient genetic diversities to differentiate the organisms in the same species or populations.

For higher accuracy and reproducibility, the DNA-based analysis of genotypes has been attempted on the basis of DNA polymorphism between or within species. Firstly adopted method was restriction fragment length polymorphism (RFLP) analysis, which reflects the DNA

alteration by point mutations within the recognition sequence as well as insertion and deletion [2]. The different restriction patterns by hybridizing DNA probes in a given sequence bring about a detectable polymorphism between different genotypes.

In order to expand the applicability of DNA probes in detecting the polymorphism, an alternative means called DNA fingerprinting (DFP) have been developed from RFLP analysis [3,4]. Instead of probe derived from a specific gene, the multilocus probes are mainly used for the detection of more or less regular arrays of tandemly repeated motifs on DNA. There are two categories of such multiloci in higher organisms; minisatellites of tandem repeats having a basic motif of 10 to 60 bases, and microsatellites of tandem repeats having very short motifs less than 5 bases. Since this kind of tandem repeats are in nature characterized by highly variable copy numbers of identical or closely related basic motifs, the concept of variable number of tandem repeats (VNTR) has been introduced for the designation of DNA polymorphism by DFP [5].

Shortly after the introduction of polymerase chain reaction (PCR) for DNA amplification, this molecular biological revolution has been also proved to be feasible for the detection of DNA polymorphism of higher organisms at mid-1980 [6]. Even though specific primers complementary to a known sequence were employed to reveal DNA polymorphism, arbitrary sequence of 10 nucleotides having at least 50% (G+C) content is now more widely used for the generation of PCR amplification products from genomic DNA, which is so-called random amplified DNA polymorphism (RAPD) analysis [7]. This methodology is superior to DFP analysis because it requires small quantity of sample DNA with its ease and rapidity and radioactively labeled probes are no more needed for analysis.

As a modified RAPD analysis, arbitrary primed-PCR (AP-PCR) method was approached for PCR amplification of genomic DNA using oligonucleotide primers longer than 20 bases in early 1990s [8,9]. In this procedure, 2 cycles of DNA amplification with low stringency at initial step, which allows some mismatches, are followed by 30 to 40 cycles of amplification with high stringency.

A very recently developed approach is the amplified fragment length polymorphism (AFLP) analysis as an indigenous combination of RFLP and RAPD analysis [10]. The restriction fragments of genomic DNA are ligated with adaptors of a defined sequence, and amplified with 5'-portion of primer complementary to the adaptor and another arbitrary primer for 3'-end extension.

All of the above methodologies for the detection of DNA polymorphism have been successfully adopted in the identification, breeding and improvement of domestic animals. In addition, DNA markers developed by those DNA technologies showed high potentials to be directly applied as simple selection markers for animals, especially in identification of breeds [11-17], selection program [15,18-21], genetic analysis [20,22], and confirmation of economic traits [23-26].

Even though the researches on the identification of Korean native animals by genetic analysis are not so much made in Korea till now, its necessity is strongly required for sustenance and preservation of their own gene sources and further for their genetic improvement. The current results from genetic analysis offered the possibility of the accurate genetic estimation and identification of Korean native animals from the other imported breeds, by means of DNA marker-assisted selection (MAS). And, the characterized DNA markers related to economic traits could be usefully applied in the selection of superior breeds as well as in further improvement of those traits.

PHENOTYPIC CHARACTERISTICS OF KOREAN NATIVE ANIMALS

As mentioned above, Korean native animals have not been well kept during Japanese colonization and industrialization period in modern ages. Fortunately, Korean native cattle (Hanwoo) has been preserved and improved by Hanwoo Improvement Center and Korea Animal Improvement Association since 1960s. However, Korean native pig and chicken have been almost eradicated in Korea.

Some articles written by Japanese in early 20th century described well the phenotypic characteristics of Korean native animals. The photographs of Korean native animals are shown in Fig. 1.

Following the paper [27], Hanwoo has temperate but endurable characters with strong adaptation capability against fodder and strong and sturdy constitution for employing in cultivation. The external shape of Hawoo lacks the elegance due to sturdy and big bones. Slow growth and tardy fatting are also pointed out in Hanwoo. However the Hanwoo beef shows good quality to Korean consumers. It is generally thought that Hanwoo has the similar historic origin with Chinese Yenbian, and Japanese Black (Wagyu).

Korean native pig was reported to have black glossy hairs, caved face, greatly protruded mouth, big eyes, straightly upright ears, round shoulder, narrow rear back, wide chest, long hip, well-balanced short legs, and 10-12 teats [28]. It takes 14 months in sexual maturation. Litter size is 6-8 piglets 2 times every year, and the piglets wean the milk after 8 weeks. The major characteristics of native pig are high propagating power, superior meat quality and strong adaptation capability, but low growth rate and tardy fatting are still problems.

Korean native chicken in literature [29] has yellowish, reddish or grayish brown-colored feathers with black stems and a single comb. Black color at abdomen was generally observed in male native chicken. The average weight of female adult is reported as 1.3-1.5 kg and male, 1.7-1.8 kg. They produced 150 eggs every year, of which average weight was 43-46 g. They were also characterized by strong healthy constitution. nimble behavior, strong flying power and broody character, but had significant drawbacks like low egg productivity, low meat content and lacking in weight.

For the analysis of genetic traits of Korean native animals, Hanwoo registered in Korean Animal Improvement Association has been generally used, and Korean native pig and chicken were selected by researchers from local farms according to phenotypic description in

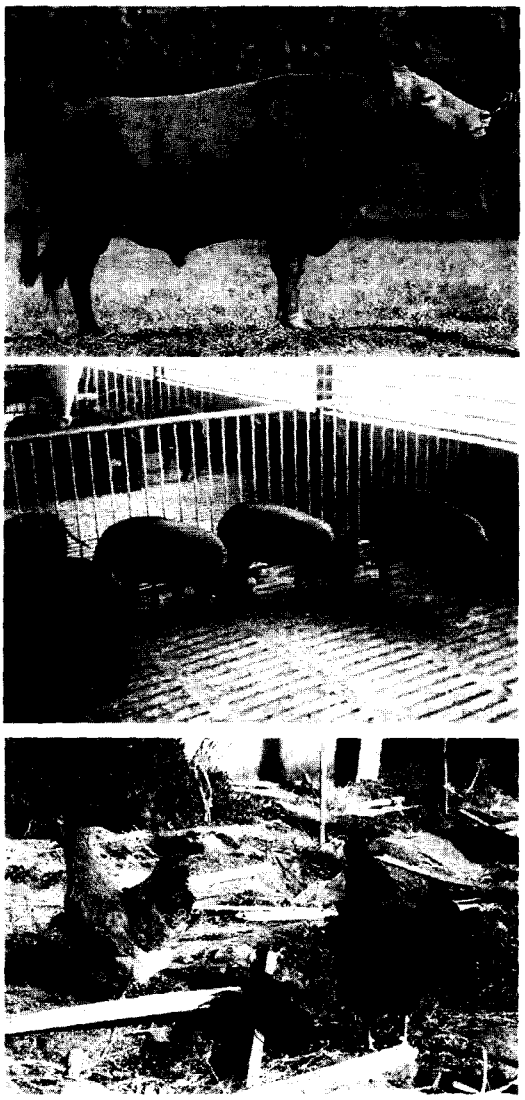


Fig. 1. The photographs of Korean native animals.

Japanese articles.

DNA MARKERS BASED ON GENETIC TRAITS OF KOREAN NATIVE ANIMALS

Under the assumption that Korean native animals have different genetic constitution from other imported breeds, some efforts to find molecular markers based on DNA polymorphism have been made in 1990s, and a few DNA marker unique in native animals was found and characterized. The selected DNA markers were successfully employed in breed identification and pedigree analysis of Hanwoo by PCR amplification [30-32,46] or by VNTR analysis [33,34], in screening of Korean native pig by DFP using VNTR [35] or microsatellites [36] sequences, and in differentiating Korean native chicken by VNTR analysis [37,38] or PCR amplification [39]. Typical examples for identification of Hanwoo and Korean native pig are shown in Fig. 2 and Fig. 3.

Proper selection and design of new specific PCR primers or microsatellite probes for VNTR which can differentiate Korean native animals distinctively from other breeds are the most important ones in their easy identification and selection [40-42]. In general, well-matched probes or primers with 17-22 nucleotides long, devoid of consecutive tracts of a single nucleotide, with a G+C

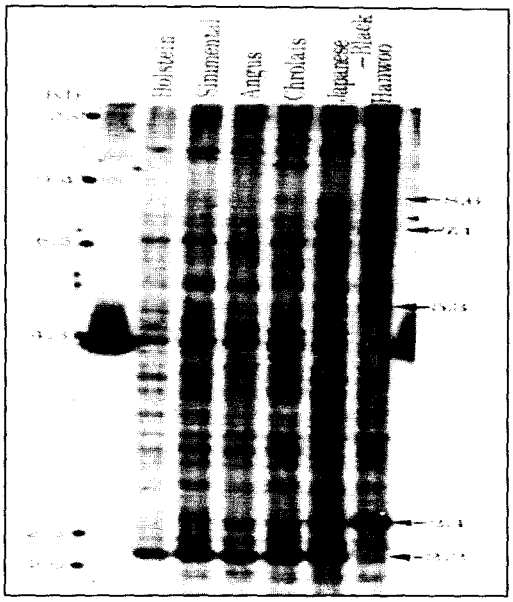


Fig. 2. DNA polymorphism of cattle breeds using M13 VNTR probe and *Pst*I enzyme [33]. The sequence of M13 VNTR probe used in DFP was 5′-GAGGGTGGCGGXTCT-3′. Arrows show the characteristic DNA bands to Korean native cattle (Hanwoo).

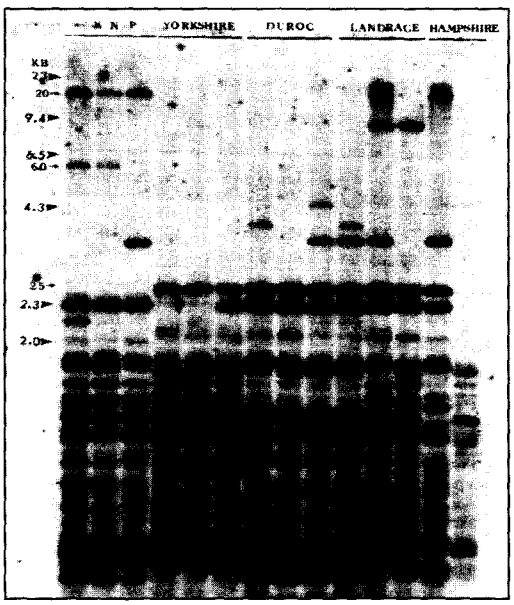


Fig. 3. DNA polymorphism of pig breeds with YNZ22 VNTR probe and *Hae*III enzyme [35]. The sequence of YNZ22 VNTR probe used in DFP was 5′-CTCTGGGTGTCGTGC-3′. Arrows indicate the characteristic DNA bands to Korean native pig.

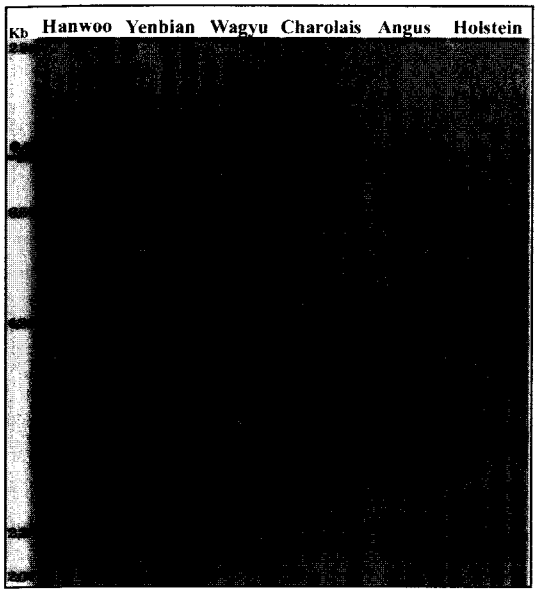


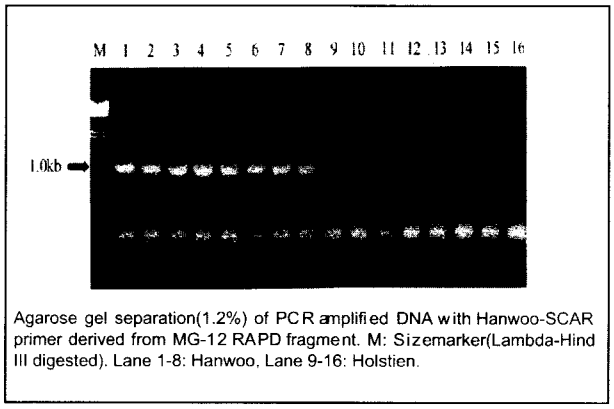
Fig. 4. Differentiated DNA markers of Korean native cattle (Hanwoo) among 6 cattle breeds using newly designed specific probe $(AAC)_n$ and *PstI* enzyme [43]. Arrows show some characteristic DNA bands to Hanwoo.

content around 50% (T_m approximately 60°C), and preferably G- or C-rich at the 3′ end are designed and employed as new DNA marker for selection. Thus the possibility as DNA probes or primers designed from several repetitive sequences such as VNTR found in Korean native animals were checked in order to differentiate Hanwoo to the other cattle breeds [43].

Fortunately one VNTR probe designed from repetitive sequence, $(AAC)_n$, showed a unique 9.4 kb DNA marker in Hanwoo distinctive from foreign cattle breeds in DFP as seen in Fig. 4. Among cattle breeds tested, including Asian breeds having the same origin with Hanwoo, one of Chinese Yenbian cattle gave the same marker pattern as Hanwoo, which implies Chinese Yenbian has the same inheritance with Hanwoo. Following this, DFP using $(AAC)_n$ probe and *Pst* I restriction enzyme was successfully adopted in identification of the genetic purity of Hanwoo populations and in accurate separation of individuals in doubt [43].

Recently Hong and his coworkers [40] reported several specific primers derived from sequence characterized amplified region (SCAR) by RAPD method. The SCAR primer designed from MG-12 RAPD fragment successfully discriminated Hanwoo from Holstein and Hereford as likely as Fig. 5.

Although the designed probes and primers could be applied for the successful discrimination of Hanwoo, the identification of all the Hanwoo individuals in field is not so easy because those are designed based on a partial character of Hanwoo DNA, and because lots of Hanwoo were already hybridized with other imported breeds after 1970s. Nevertheless, the application of those DNA probes and primers will be valuable in improvement scheme of Hanwoo by distinguishing genetically



Agarose gel separation(1.2%) of PCR amplified DNA with Hanwoo-SCAR primer derived from MG-12 RAPD fragment. M: Sizemarker(Lambda-Hind III digested). Lane 1-8: Hanwoo, Lane 9-16: Holstien.

Fig. 5. Discrimination of Korean native cattle (Hanwoo) from Holstein by RAPD using newly designed SCAR (sequence characterized amplified region) primer [40]. Arrow indicates the characteristic DNA band to Korean native cattle (Hanwoo).

pure populations from genetically hybridized individuals. In other words, the designed specific primers or probes will be applied for the identification of specific DNA markers in the improvement strategies for Hanwoo, through the identification and preservation of pure populations by systematic registration for breeding.

DNA MARKER-ASSISTED SELECTION (MAS) OF KOREAN NATIVE ANIMALS

Until now the popular breeding scheme for major valuable traits of domestic animals were selection of individuals or family based on statistical probability of phenotypes, and eventually the progress of their genetic improvement went to plateau. In case of Hanwoo, selection criteria were put on the improvement of daily gain and marbling as the most important economic traits, but those criteria have some limitations in sufficient reflection of environmental effects such as management and castration, and in estimation of the effect on the statistical probability. Korean native pig has some critical defects including difficulty to increase growth and reduce fat content, but it produces superior quality of meat favorite to Korean consumers. Low egg productivity and slow growth of Korean native chicken is one cause of extinction.

In order to overcome some problems in traditional breeding system, new strategy is required based on DNA polymorphism related to economic traits. As a powerful tool to improve the performance of Korean native animals, the development of molecular discrimination markers is recently emphasized for the identification of breeds having economic traits. Recently, the relationship between traits and DNA markers has been reported, for example, daily gain and marbling of Hanwoo [42,43], growth and porcine stress syndrome (PSS) of pig [44,45], and egg productivity of chicken [41]. Now we are in beginning era of DNA marker-assisted selection (MAS) of Korean native animals for their improvement by simple and accurate analysis of breeds.

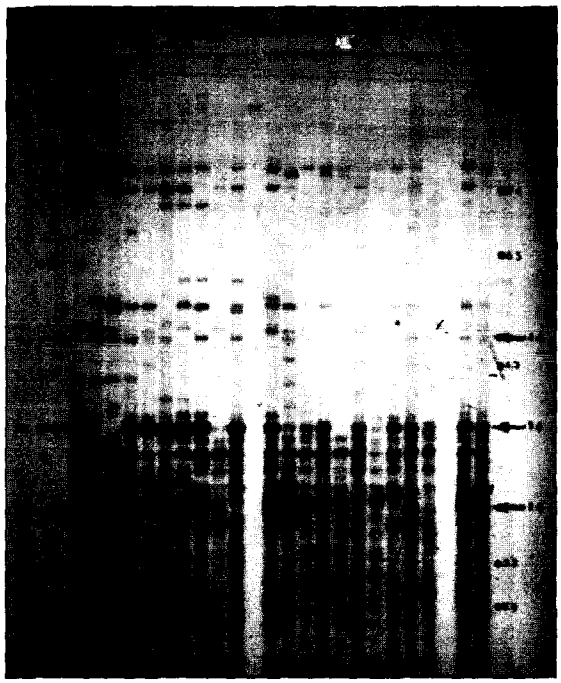


Fig. 6. DNA markers related to growth rate of Korean native cattle (Hanwoo) using M13 VNTR probe and *Hae*III enzyme [43]. The sequence of M13 VNTR probe used in DFP was 5'-CAGGGTGGCGGXTCT-3'. Arrows indicate the specific DNA markers related to daily gains.

Korean Native Cattle (Hanwoo)

The economic traits of Hanwoo, the most important genetic source in Korea, have been considered as growth rate and marbling in beef. When 3 specific DNA markers by DPF with M13 VNTR probe (5'-GAGGGTGGC-GGXTCT-3') were correlated with growth rate of Hanwoo, one gave negative effect and two showed positive on daily gain, but the combined effect of any DNA markers were observed to be also positive, as shown in Fig. 6 [42,43]. Upon introducing statistical modification of market month and sex (bull and steer) for individual, least square mean value of growth was 730-735 g per day for group showing a negative marker, whereas daily gain value as high as 790-794 g was achieved in populations having any of two positive markers or any combined marker groups (Table 1). This suggests that increase of body weight of Hanwoo can be gained as much as 40 kg (60 g difference during 700 days) before market by removing individuals showing the negative marker. Likely this, any economic traits of Hanwoo can be correlated with DNA markers in DFP using any specific DNA probes, and then applied in breed improvement.

Marbling is one of Korean conclusive factors to decide beef quality and price, and provides the differentiation of Hanwoo from imported beef by Korean consumers in the open market. When 2 specific DNA markers from AFLP were correlated with marbling score by Korean grading system, which classifies beef grades in 3 degrees [43], higher distribution of specific markers was observed in Grade 1, and lower proportion in Grade 3, as shown in Table 2. Individuals showing both markers were characterized by high marbling score, as likely as only 3% in Grade 3, whereas the groups without any DNA markers produced more than 30% of beef in Grade 3. The possibility to have beef in Grade 1 was found to be double in the group giving 2 specific markers in AFLP (81.2%), compared to the group without both markers (42.8%). Because the selection of marbling has limitation for a long time owing to checking at slaughter but not at live, this kind of selection by DNA markers is epochal to improve marbling of Hanwoo at live state.

Table 1. Least square mean values of daily gains correlated with DNA markers of bull and steer of Hanwoo appeared in DFP using M13 probe and *Hae*III restriction enzyme [42]

Markers (kb)	4.6	×	O	×	×	O	O	×	O	Total
	3.6	×	×	O	×	O	×	O	O	or
	2.8	×	×	×	O	×	O	O	O	Mean
No. of head		18	8	49	34	36	27	59	56	287
Daily gain (g)		735	731	794	777	777	790	785	788	781
±SE		20.5	28.1	15.2	17.5	17.2	17.9	15.5	15.5	14.2

O : Specific DNA markers were observed in DFP using M13 probe and *Hae*III enzyme, × : Specific DNA markers were not observed in DFP using M13 probe and *Hae*III enzyme, SE : Standard error.

Table 2. Distribution of marbling grades in Korean native cattle (Hanwoo) groups showing DNA markers when AFLP analysis was employed with E2 and T3 primers [1] [43]

Marker (bp) / Grade	330	210	330 and 210	None	Total
3	4 (10.3%)	1 (6.3%)	1 (3.1%)	10 (35.7%)	16 (13.9%)
2	10 (25.6%)	3 (18.8%)	5 (15.7%)	6 (21.3%)	24 (20.9%)
1	25 (64.1%)	12 (75%)	26 (81.2%)	12 (42.8%)	75 (65.2%)
Total (Mean)	39 (10.85[a])	16 (11.85[a])	32 (12.46[a])	28 (8.41[b])	115 (10.74)

[1] E2 primer; 5'-GACTGCGTACCAATTCAAC-3', T3 primer; 5'-GATGAGTCCTGACCGAAAG-3'

Korean Native Pig

Korean native pig has been almost extinct due to weakness for growth, but recently the meat quality is recognized to be preferable to Korean consumers. Yeo and his colleagues [44] found some DNA markers related to daily gain and backfat thickness by means of AFLP. The individuals showing DNA marker at 270 bp attained the delayed growth of 40 g per day, compared to other groups having 279 bp marker or without both markers (Table 3). Backfat of pigs after 6 month was 1.5 mm thinner in the group having 290 bp marker, compared to the group showing 370 bp marker or without both markers.

Table 3. Correlation of DNA marker distribution with daily gain and backfat thickness of Korean native pig when AFLP method was applied [44]

E3 and T2 Primer [1]	DNA Marker (bp)				Mean
	270	279	270 and 279	none	
Body weight at birth (Kg)	1.7	1.3	1.4	1.5	1.4
Body weight at 6 month (Kg)	62.3	55.6	60.5	60.7	59.8
Daily gain (g)	347	309	336	349	329
E4 and T4 Primer [2]	DNA Marker (bp)				Mean
	290	370	290 and 370	none	
Backfat thickness at 6 month (mm)	12.05	13.55	13.09	13.57	13.00

[1] E3 primer; 5'-GACTGCGTACCAATTCAAG-3', T2 primer; 5'-GATGAGTCCTGACCGAAAC-3'

[2] E4 primer; 5'-GACTGCGTACCAATTCACA-3', T4 primer; 5'-GATGAGTCCTGACCGAACA-3'

Table 4. The mean value differences in economic traits between two homozygotes (NN and nn) and between heterozygote (Nn) and homozygotes (NN and nn) of porcine stress syndrome genotyre, which were determined by PCR-RFLP method [45]

Trait	NN-nn [1]	Nn - (NN + nn)/2 [2]
Age at 30 Kg (day)	-4.2	-0.7
Average daily gain (g)	78.9	34.45
Age at 90 Kg (day)	-9.3	-2.75
Feed efficiency	-0.05	-0.035
Backfat thickness (cm), male	0.055	0.0165
Backfat thickness (cm), female	-0.068	-0.057
Lean meat content (%)	-0.03	0.05

[1] NN - nn = the mean value difference between dominant homozygote (NN) and recessive homozygote (nn).

[2] Nn - (NN+nn)/2 = the mean value difference between heterozygote (Nn) and two homozygotes (NN and nn).

The porcine stress syndrome (PSS) genotype was also determined by PCR-RFLP method [45]. The superiority of average daily gain of dominant (NN) pigs to recessive (nn) pigs was estimated as 78.9 g, and heterozygosis (Nn) of this genotypes showed half value of dominant (NN) ones in the difference of average daily gain (Table 4). Besides, any significant effects were not observed in other traits.

Korean Native Chicken

The important economic traits of Korean native chicken are body weight, egg weight and egg production. The correlation of those traits with DNA markers

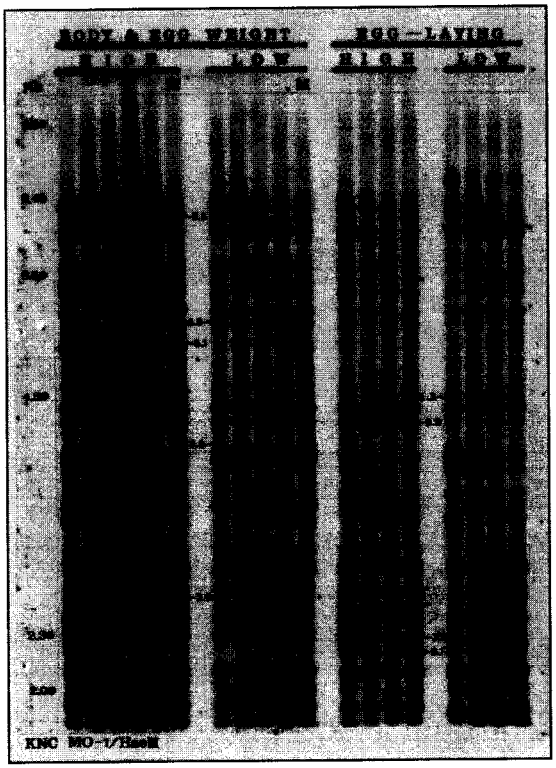


Fig. 7. DNA fingerprinting of Korean native chicken divided two groups using Mo-I probe and *Hae*III enzyme [41]. The sequence of Mo-I probe used in DFP was 5'-TGCCCAGTC-CTCCC-3'. Arrows show some economic specific markers in Korean native chicken.

in DFP using VNTR probe revealed some variable polymorphism of DNA fragments in Korean native chicken of high and low performances (Fig. 7) [41]. Especially the results from Mo-I or M13 VNTR probe with *Hae*III enzyme showed the possibility of application in identification and improvement of Korean native chicken.

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