Establishment of an Individual Identification System Based on Microsatellite Polymorphisms in Korean Cattle (Hanwoo)

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ABSTRACT : This study was conducted to establish an individual identification system comprising of 19 microsatellite markers located on different bovine autosomes. The markers were typed on 257 animals from five cattle breeds. In total, 112 alleles were detected from the genotyping of 19 microsatellite markers. The average heterozygosities ranged from 0.292 to 0.824 and the polymorphic information content (PIC) ranged from 0.274 to 0.817 in Hanwoo. We found that there were differences in allele frequencies in Hanwoo when compared with other cattle breeds. The calculated cumulative power of discrimination (CPD) was 99.999% when nine microsatellite loci were used for analysis in the individual identification system. Also the matching probability, the probability that two unrelated animals would show the same genotypes, was estimated to be 0.44×10^{-9}. Therefore, the nine markers used in this study will be used for individual identification in two million Hanwoo individuals. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 6 : 762-766)***

Key Words : Individual Identification, Microsatellites, Polymorphic Information Content, Cattle

INTRODUCTION

The individual identification system has been widely used for breeding purposes in livestock animals. In the case of breeding stocks, correct individual identifications are essential since their abilities were directly passed down to the next generation. The early method of individual identification in cattle was use tattoos and ear tags that contained individual identification numbers. These have been gradually replaced with electronic chips which have more individual data on them (Seo et al., 2001). Recently the identification system included blood typing results based on the protein polymorphisms for parentage testing and to determine whether they matched with the individual information from the ear tags and electronic chips. Because of the experimental complexity of the blood typing system, blood typing had been replaced with DNA testing, which was already widely used in the forensic sciences on human (Lee et al., 2004).

Considering all the factors involved in beef production, individual identification using DNA testing is the most appropriate solution to give all the breeders' information to the consumers. However, correct records of the animals and their products should be available before individual identification by using DNA testing. DNA test would also help for breed identification. In the Korean beef marker situation, mislabeled beef products appear in the markets because of the high prices of Korean cattle (Hanwoo beef). In comparison with the simple RFLP testing that was used for distinguishing between Hanwoo and Holstein, an individual identification needs more complex statistical procedures and for doing this, the establishment of a nation-wide analysis system is needed. In the case of Japan, an individual identification and paternity control system for Japanese Black cattle using DNA markers had been developed and used (Hirano et al., 1996). Generally speaking, individual cattle was its own genotype except for identical twins. These genetic diversities among individuals arose from mutations that accumulated over time (Evett et al., 1998). The best known DNA markers for individual cattle identification are microsatellite markers which have large number of alleles and wide distribution among the chromosomes (Gillespie et al., 1998). For the individual identification test, the power of discrimination (PD) is calculated from the genotype frequencies of the selected microsatellite markers for minimizing the possibility of having the same genotypes in two different animals (Chen et al., 2004; Cho et al., 2004).

However the individual identification test based on microsatellite markers also have known experimental errors for decoding the analogue information. In order to have high testing power and reliable confidence for the individual identification test in Korean cattle population, microsatellite markers should be carefully selected. Therefore, the aim of this study is to select the best microsatellite markers for the individual Korean cattle identification test and to apply the DNA test to trace the

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Table 1. Breed, number of individuals and origin of the cattle used in this study

Breed	No	Origin
Hanwoo (HW)	131	Korea
Angus (AN)	38	Scotland
Hereford (HF)	33	English
Charolais (CH)	35	France
Holstein (HOL)	20	Netherlands
Total	257	

movement of the beef products in the markets.

MATERIALS AND METHODS

Animals and microsatellite markers

The cows used in this study to analyse and characterize microsatellite markers belong to four foreign breeds and Hanwoo. Eighteen markers developed by ILRI (International Livestock Research Institute) and recommended by ISAG (International Society for Animal Genetics) for individual traceability and identification were used in this study.

PCR and genescan

PCR was conducted with a final volume of 10 μl, including 1 μl of 10×reaction buffer (10 mM Tirs, pH 8.3, 50 mM KCL, 0.1% Triton ×100, 1.5 mM $MgCL_2$), 0.7 μl dNTP Mix (2.5 mM), 10 pM of each primer, 20 ng of genomic DNA, and 0.5 U of Taq polymerase. Amplification of PCR products was carried out using a standard PCR program with 5 min denaturation at 94°C, 30 cycles for 30 sec at 94°C, 30 sec annealing at 55-65 °C, 1 min extension at 72°C, and final extension for 10min at 72°C. PCR product 1 μl was mixed with 0.5 μl GS400 TAMRA size standard (DNA fragments of known size labeled with ABI PRISM dye *N, N, N*^{l}-tetra-methyl-6-carboxy-rhodamine (TAMRA)) (Perkim-Elmer, USA) and 8 μl loading formamid solution. The samples were denaturated by heating at 90°C for 5 min followed by cooling on ice. Analyses of PCR products were performed by using the ABI 3100 Genetic Analyzer (Applied Biosystems, USA). The relative ratios of the detected virus sequences were determined by comparison of the peak area value fork of each of the detected fragments.

Statistical analysis

The MS toolkit software (Kim, 2000) was used to estimate the heterozygosity frequency and marker allele frequency. The Polymorphic Information Content (PIC) of Nei (1972, 1978) was used for each microsatellite locus.

$$PIC = 1 - \sum_{i=1}^{n} P_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} P_i^2 P_j^2$$

Table 2. Characterization of 18 microsatellite loci analyzed in five cattle breeds

Locus	Chromosome location	Size range (bp)	No. of alleles	Ht
ILSTS005	10	180-196	9 (4)[a]	0.530
ILSTS006	7	275-303	13 (6)	0.809
ILSTS008	14	171-189	10 (5)	0.541
ILSTS013	9	116-134	10 (5)	0.731
ILSTS023	17	163-203	15 (5)	0.724
ILSTS028	11	129-163	18 (3)	0.657
ILSTS050	2	140-170	16 (6)	0.835
ILSTS103	21	184-262	16 (6)	0.794
MGTG4B	4	129-167	16 (11)	0.815
TGLA122	21	134-190	27 (8)	0.862
TGLA126	20	111-129	10 (7)	0.715
TGLA227	18	78-106	6 (4)	0.719
BM1824	1	178-194	9 (6)	0.706
BM2113	2	116-150	14 (7)	0.836
ETH10	5	210-226	9 (6)	0.767
ETH225	9	138-168	13 (6)	0.801
ETH3	19	103-131	12 (6)	0.714
SPS115	15	245-261	9 (7)	0.721

[a] Number of alleles that is found in Hanwoo population.
Ht: Expected total heterozygosity.

where n = number of alleles; P_i = frequency of ith allele; and P_j = frequency of jth allele. Power to Discriminate (PD) was computed as a probability that two randomly chosen individuals do not have the same genotype. Matching probability (W) was computed as:

$$PD = 1\text{-}W$$

$$PD = 1 - \sum_{i=2}^{k} X^2_i$$

where k = number of genotypes in a given population and *Xi* = frequency of i^{th} genotype at a particular locus. Cumulative PD (CPD) was computed as:

$$CPD = 1 - \prod_{i=1}^{M} (1\text{-}PD)$$

where M is the number of marker loci tested.

RESULTS AND DISCUSSION

Heterozygosities for 18 microsatellite markers were calculated in five cattle breeds which have different genetic backgrounds. The identified allele numbers were from 6 for locus TGLA227 to 27 for locus TGLA112. The calculated heterozygosities for the five populations were between 0.54 to 0.84. The varied heterozygosities indicated that markers with high levels of heterozygosities were needed for individual identification or parentage testing. This is especially important, in order to have a high accuracy individual identification system for Hanwoo cattle. The

Table 3. Estimates of heterozygosity (H) and polymorphic information content (PIC) in five cattle breeds

Locus	HW		AN		HF		CH		HOL	
	H	PIC	H	PIC	H	PIC	H	PIC	H	PIC
ILSTS005	0.500	0.449	0.458	0.427	0.079	0.078	0.304	0.294	0.401	0.361
ILSTS006	0.756	0.745	0.724	0.719	0.787	0.785	0.845	0.838	0.760	0.741
ILSTS008	0.610	0.602	0.239	0.224	0.497	0.450	0.553	0.167	0.521	0.466
ILSTS013	0.706	0.678	0.590	0.556	0.700	0.681	0.672	0.641	0.401	0.361
ILSTS023	0.678	0.650	0.891	0.891	0.836	0.833	0.878	0.877	0.894	0.894
ILSTS028	0.292	0.274	0.462	0.450	0.551	0.540	0.623	0.587	0.682	0.659
ILSTS050	0.663	0.637	0.663	0.640	0.751	0.733	0.697	0.675	0.833	0.820
ILSTS103	0.592	0.569	0.564	0.553	0.758	0.744	0.602	0.581	0.683	0.653
MGTG4B	0.758	0.747	0.733	0.718	0.732	0.709	0.734	0.718	0.725	0.704
TGLA126	0.797	0.781	0.685	0.661	0.684	0.662	0.648	0.622	0.601	0.558
TGLA122	0.824	0.817	0.962	0.962	0.849	0.848	0.847	0.847	0.924	0.924
TGLA227	0.728	0.703	0.742	0.719	0.493	0.477	0.634	0.607	0.536	0.507
BM1824	0.642	0.607	0.724	0.699	0.569	0.540	0.752	0.732	0.741	0.718
BM2113	0.737	0.716	0.809	0.796	0.712	0.699	0.763	0.748	0.726	0.704
ETH10	0.778	0.761	0.494	0.463	0.675	0.650	0.071	0.070	0.567	0.550
ETH225	0.727	0.708	0.785	0.769	0.656	0.637	0.646	0.629	0.728	0.709
ETH3	0.771	0.753	0.711	0.683	0.509	0.452	0.775	0.757	0.601	0.577
SPS115	0.750	0.735	0.632	0.601	0.582	0.557	0.645	0.615	0.611	0.587

HW: Hanwoo, AN: Angus, HF: Hereford, CH: Charolais, HOL: Holstein.
H: Heterozygosity.
PIC: Polymorphic information content.

Table 4. Estimation of matching probability (W) and power to discriminate (PD) based on 9 different markers in Hanwoo

Loci	No. of allele	Matching probability (W)		Power to discriminate (PD)	
		IW	CW	PD (%)	CPD (%)
M_1 (ILSTS006)	6	0.095	0.095	90.5	90.500
M_2 (ILSTS013)	5	0.142	0.14×10^{-1}	85.8	98.651
M_3 (TGLA122)	7	0.044	0.60×10^{-3}	95.5	99.940
M_4 (TGLA126)	4	0.072	0.44×10^{-4}	92.8	99.995
M_5 (TGLA227)	7	0.123	0.53×10^{-5}	87.7	99.999
M_6 (BM2113)	6	0.111	0.60×10^{-6}	88.9	99.999
M_7 (ETH10)	6	0.083	0.50×10^{-7}	91.7	99.999
M_8 (ETH225)	5	0.081	0.40×10^{-8}	91.9	99.999
M_9 (ETH3)	6	0.111	0.44×10^{-9}	88.9	99.999

markers chosen have to show different allele frequencies in order for Hanwoo to be distinguishable from other cattle breeds. In Table 2, the identified number of alleles for the 18 microsatellite markers in Hanwoo were between 3 and 11. Therefore, markers having 7 to 8 alleles need to be selected for individual identification. This means 16 microsatellite markers having heterozygosity values over 0.6 in Table 2 can be used as candidate markers for the individual identification system.

In order to determine the optimum markers for the individual identification system, alleles for the selected markers should be widely distributed. However most microsatellite marker alleles show distinct allele types for the various breeds. For example, a few markers, recommended for the parentage test, can be only used in Holstein breeds. Therefore we have to select our own useful markers for the individual identification system in Hanwoo. In order to evaluate the markers suitable for the individual identification system, the heterozygosities and the polymorphic information content (PIC) were calculated for the 18 markers in the five breeds (Table 3). The ILSTS028 locus showed relatively low heterozygosity and PIC in Hanwoo compared with the other 4 breeds. This indicates that the ILSTS028 marker has very limited number of alleles in Hanwoo and cannot be used as a marker for Hanwoo individual identification. The results also indicate that each marker has a different number of alleles in the different breeds and markers for the individual identification test have to be selected based on the breed specific allele patterns.

The allele frequencies for the microsatellite markers recommended by the International Society for Animal Genetics (ISAG) were investigated in five cattle breeds (Figure 1). In case of the ETH10 locus, a total of 9 alleles were identified and the allele frequencies for the 218 bp allele in Angus, Holstein, Charolais and Hanwoo were 0.658, 0.150, 0.963 and 0.287, respectively. This indicates that there are variations in allele frequencies among the

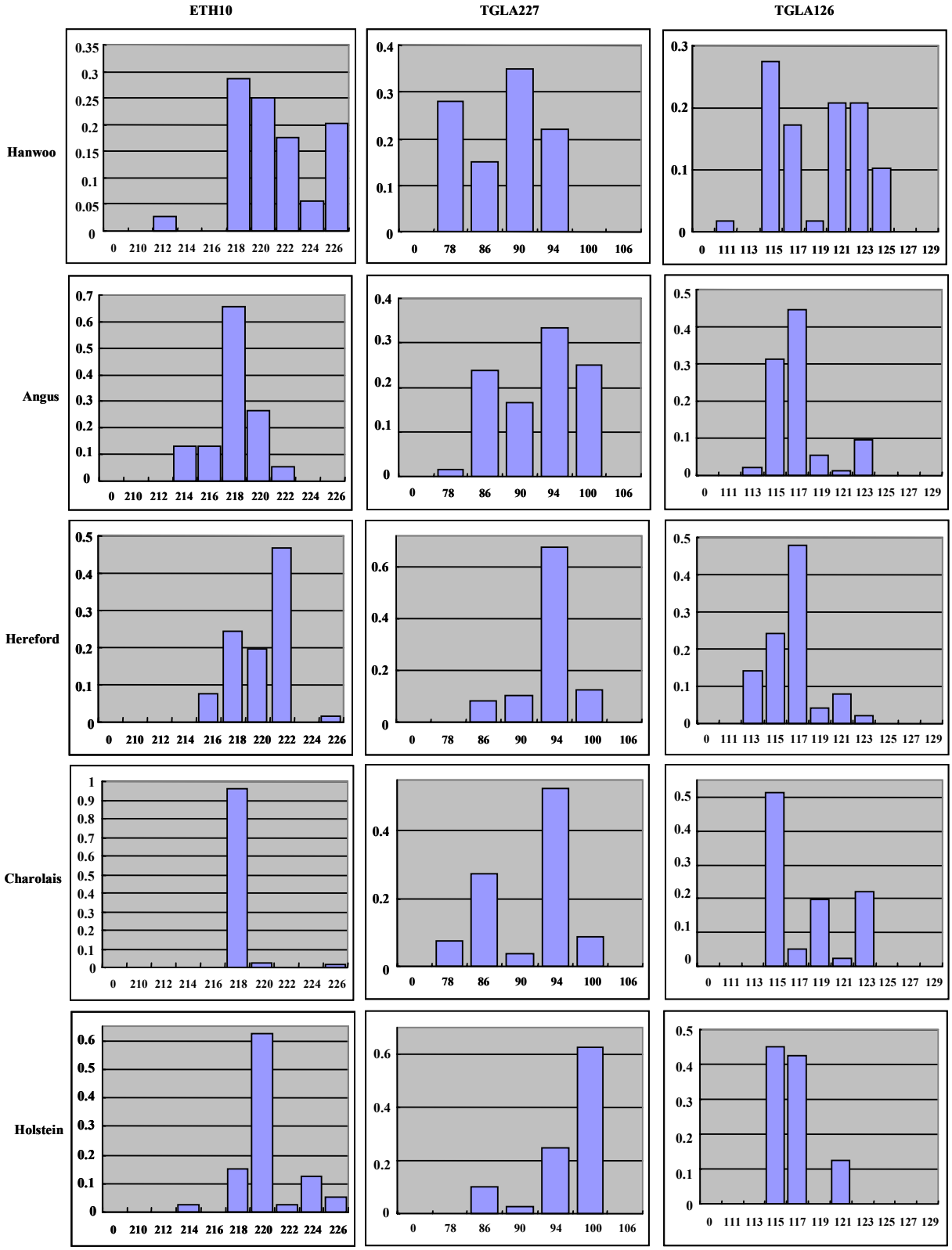

Figure 1. Allele frequency distributions for three loci five breed sample.

breeds. The different allele frequencies for the 220, 214 and 210 alleles among the breeds also support this evidence. Loftus et al. (1999) also mentioned earlier that the allele frequencies were different among the breeds. Therefore, the choice of microsatellite markers, based on a consideration of the allele frequencies among the breeds, is very

important for the success of an individual identification test.

The individual identification power and the cumulative individual identification power were estimated for the 9 candidate markers in Hanwoo (Table 4). The estimates indicates the power to discriminate (PD), the probability that two unrelated animals show different genotypes, and the PD was compared with matching probability (W), the probability that two unrelated animals show the same genotypes. In case of using 5 markers, the estimated probability that two unrelated animals show the same genotypes was 0.53×10^{-5}. When all 9 markers were included for individual identification, the estimated matching probability (W) was 0.44×10^{-9}. These results showed relatively less power to discriminate values when compared with the 0.72×10^{-6} for five microsatellite markers reported by French researchers (SanCrostoval et al., 2000). This means that the power of discrimination can be increased when the selected microsatellite markers have more effective alleles. When 23 microsatellite markers were used on the Japanese Black Cattle for individual identification, 31 trillion individual animals could be distinguished (Inoue-Murayama et al., 1997). In other words, when more markers were used for the individual identification, the probabilities that two unrelated animals show different genotypes would be increased. However, the cost and time for the analysis would also be increased when the number of markers was increased. Therefore selection of optimum markers is essential. In conclusion, the 9 markers used in this study will be appropriate for use in the individual identification of 2 million Hanwoo cattle.

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