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Individual Identification using The Multiplex PCR with Microsatellite Markers in Swine

Lee-Kung Kim[a], Chang-Min Park[a], Sun-Ae Park, Seung-Chang Kim, Hoyoung Chung, Han-Ha Chai, Gyeong-Yong Jeong and Bong-Hwan Choi[†]

Division of Animal Genomics & Bioinformatics, National Institute of Animal Science, RDA, Suwon 441-706, Republic of Korea

ABSTRACT

The swine is one of the most widespread mammalian throughout the whole world. Presently, many studies concerning microsatellites in swine, especially domestic pigs, have been carried out in order to investigate general diversity patterns among either populations or breeds. Until now, a lot of time and effort spend into a single PCR method. But simple and more rapid multiplex PCR methods have been developed. The purpose of this study is to develop a robust set of microsatellites markers (MS marker) for traceability and individual identification. Using multiplex-PCR method with 23 MS marker divided 2 set, various alleles occurring to 5 swine breed (Berkshire, Landrace, Yorkshire, Duroc and Korea native pig) used markers to determine allele frequency and heterozygosity. MS marker found 4 alleles at SW403, S0227, SWR414, SW1041 and SW1377. The most were found 10 alleles at SW1920. Heterozygosity represented the lowest value of 0.102 at SWR414 and highest value of 0.861 at SW1920. So, it was recognized appropriate allele frequency for individual identification in swine. Using multiplex-PCR method, MS markers used to determine individual identification biomarker and breed-specific marker for faster, more accurate and lower analysis cost. Based on this result, a scientific basis was established to the existing pedigree data by applying genetics additionally. Swine traceability is expected to be very useful system and be conducted nationwide in future.

(Key words : Microsatellite marker, Swine, Multiplex PCR, Individual identification, traceability)

INTRODUCTION

The swine (*Sus scrofa domestica*) is one of the most widely widespread domestic animal species in the world. It is supposed that in the history of pig domestication, complex factors such as men-deriving forces and natural selection have influenced the actual diversity and population structure of the species (Amaral *et al*., 2011). Its domestic pig is of economic importance and the present day varieties are the result of multiple domestication events that occurred in different regions. And genetic diversity at swine breed is impossible to individual identify. One case changed foreign and domestic pigs were frequently. In addition, these case scientific forensic techniques are nonexistent situation to prevent method of currently disguised distributors. So, development of DNA marker techniques based on swine genetic is required. Moreover, these characteristics are the recent increasing interest for traceability system of the meat can be used. Traceability of cow was most research activity done. However, studies related to the traceability of swine in the country were insufficient. Additionally, gene profiling studies utilizing of mitochondria DNA, blood protein, minisatellite, microsatellite, single nucleotide polymorphism (SNP) and various genetic markers by the recognition of the value of conservation and utilization in many traditional livestock as genetic resources in various worlds carried out in the traditional livestock (Chung *et al*., 2001; Girish *et al*., 2007; Ichikawa *et al*., 2001; Kim *et al*., 2010; Oh M. Y. *et al*., 1992; Signer and Jeffreys, 1997). In particular, the microsatellite has a regular repeating sequence of 2~6 base pairs of DNA, are distributed approximately 50,000 to 100,000 across the entire mammalian genome and is non-coding DNA sequence of the wide range of high polymorphism (Debrauwere *et al*., 1997). It is widely used to analysis of the genetic diversity of livestock populations because of convenience and polymorphism of the experiment among the technology using DNA (Bar-

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[a] These authors contributed equally to this work.

[†] Corresponding author : Phone: +82-31-290-1592, E-mail: bhchoi@korea.kr

ker *et al*., 1997; Bjornstad *et al*., 2003; Laval *et al*., 2000; Li *et al*., 2000). Currently, multiplex PCR as well as simple PCR technique using microsatellite marker (MS marker) are also being developed to save time and costs (Jamsari *et al*., 2011; Koskinen and Bredbacka, 1999; Weissenberger *et al*., 2010).

Microsatellites can be used to investigate the population structure, including the genetic relationships, among subpopulations. This study examined to determine gene identification and individual identification through traits associated biomarkers and breed- specific marker for faster, more accurate and ways to reduce the analysis cost using optimized multiplex-PCR method with known MS marker.

MATERIALS AND METHODS

Animals and Extraction of DNA from Blood

DNA was extracted from blood of total 96 by 5 swine at each breed (Berkshire, Landrace, Yorkshire, Duroc and Korea native pig,) using Wizard genomic DNA purification kit (Promega, USA) and analyzed concentration and purity at absorbance of 260 nm and 280 nm using ND-1000 spectrophotometer (Nanodrop, USA).

MS Marker for Allele Analysis

MS markers utilized this study were firstly selected 511 MS markers based on microsatellite genetic loci of

Table 1. List of microsatellite markers and primer

Set No.	CHR	Name	Primer		Tempe-rature	Product size	Dye
			Foward(5′→3′)	Reverse(3′→5′)			
Set 01	16	SW403	GTGTATGTTCATGCATGGGTG	GTCTCTGCTTTGCTTGCATG	60℃	100～114	Fam
	2	S0226	GGTTAAACTTTTNCCCCAATACA	GCACTTTTAACTTTCATGATGCTCC		175～206	Fam
	14	SW210	TCATCACCATCATACCAAGATG	AATTCTGCCAAGAAGAGAGCC		215～250	Fam
	8	SW2410	ATTTGCCCCCAAGGTATTTC	CAGGGTGTGGAGGGTAGAAG		102～124	VIC
	4	S0107	CAAGGATGCCTGTAACTGGTGCAG	TCCTTAAGGCCTCGTAGGATCTGT		165～190	VIC
	4	S0227	GATCCATTTATAATTTTAGCACAAAGT	GCATGGTGTGATGCTATGTCAAGC		225～256	VIC
	18	SWR414	GATTTGACCCCATGCCTG	AAGGCAAACCCCTTGAGTTC		138～158	NED
	13	S0068	AGTGGTCTCTCTCCCTCTTGCT	CCTTCAACCTTTGAGCAAGAAC		211～260	NED
	17	SW1920	GATCCGTATCTATAGCCACCTG	ATGAAAGCTACCAACCCTTCC		90～135	PET
	2	SWR2516	GTGCATTATCGGGAGGTATG	ACCCTGTATGATACTGTAACTCTGG		154～178	PET
	7	S0101	GAATGCAAAGAGTTCAGTGTAGG	GTCTCCCTCACACTTACCGCAG		197～216	PET
Set 02	5	S0018	GCACAGTTGATGCTTCATGC	GATCAAAAGTCCCCAATTCC	61℃	248～277	NED
	10	SW1041	ATCAGAAAATGGTCAACAGTTCA	GGAGAATTCCCAAAGTTAATAGG		93～103	PET
	11	SW1377	TTCAAGGTTGGAAAGACAGTCC	ATGAGGAGTTTGAACTATTGGG		205～228	NED
	14	SW1557	TTCAAGGTTGGAAAGACAGTCC	ATGAGGAGTTTGAACTATTGGG		84～100	NED
	15	SW1989	TGCTCTAATCTACCCGGGTC	CCACCCCACTCCCTTCTG		228～243	Fam
	9	SW2401	TGAACAAGTCCAACCAAGAGC	CCCAACTAACGGGCTTGTG		148～170	Fam
	X	SW2456	GAGCAACCTTGAGCTGGAAC	AATGTGATTGATGCTGTGAAGC		189～211	PET
	14	SW2515	CCATCTCATCCAGAAACATCC	AGGATGCTGAGGTGTTAGGC		90～108	Fam
	6	SW316	TTCTCCAGCCATCATGAGTG	AATGACCATTCCTGAGGCTG		133～159	PET
	5	SWR1526	CGGTGGCTACAGATAACAATAC	ATCCGATTCAACCCCTAGC		114～146	VIC
	10	SWR1849	CCTGTTCTGCCTCTAGCCTG	CTGAGAAGCCTGTGCATCAG		115～160	NED
	13	SWR1941	AGAAAGCAATTTGATTTGCATAATC	ACAAGGACCTACTGTATAGCACAGG		202～222	VIC

swine reported Mapviewer database of NCBI (National Center for Biotechnology Information) and were selected 23 MS markers considered annealing temperature of 61℃, product size and type of dye for Gradient PCR thereafter. The selected MS markers divided into 2 set of each 12 and were composed of the final set of 11 and 12 that satisfied condition of multiplex PCR. Total 23 primers of 2 set are shown in Table 1.

Multiplex PCR and MS Analysis

Multiplex PCR was set up in 25 ul reaction volume consisting 6 ul (20ng/ul) of genomic DNA, 0.4 ul (10 pmole) each of fluorescence dye primer set (forward and reverse), 1 ul (Unit/ul) of Hot Start Taq DNA polymerase, 4 ul of 10× buffer and 3 ul of 2.5 mM dNTP. Conditions of Thermal Cycler PTC-0240 (MJ Research, Inc., MA, USA) were as follows: 15 min at 95℃ for initial denaturation, followed by 5 cycles with denaturation at 94℃ for 40 sec, annealing at 61℃ for 40 sec and elongation at 72℃ for 40 sec, 5 cycles with denaturation at 94℃ for 40 sec, annealing at 60℃ for 40 sec and elongation at 72℃ for 40 sec, 25 cycles with denaturation at 94℃ for 40 sec, annealing at 59℃ for 40 sec and elongation at 72℃ for 40 sec. The final had a extension temperature of 72℃ 20 min. PCR products were analyzed using the ABI-3730XL genetic analyzer (Applied Biosystems, USA) and GeneMapper version 4.0 (Applied Biosystems, USA).

Statistic Analysis

Table 2. Allele and heterozygosity at microsatellite marker

Set No.	MS marker	Allele size	Allele (Total)	Allele (K)	Allele (B)	Allele (L)	Allele (Y)	Allele (D)
Set 01	SW403	100~114	4	2	3	3	4	2
	S0226	175~206	5	4	5	5	3	2
	SW210	215~250	8	8	6	5	3	3
	SW2410	102~124	6	1	1	4	3	3
	S0107	165~190	9	3	4	5	5	3
	S0227	225~256	4	2	2	2	1	3
	SWR414	138~158	4	1	3	2	2	3
	S0068	211~260	9	3	6	5	3	6
	SW1920	90~135	10	3	6	5	3	6
	SWR2516	154~178	7	2	6	4	4	2
	S0101	197~216	7	2	4	4	4	3
Set 02	S0018	248~277	6	2	3	4	3	4
	SW1041	93~103	4	3	2	2	4	2
	SW1377	205~228	4	3	3	2	2	2
	SW1557	84~100	6	2	3	6	5	4
	SW1989	228~243	8	3	5	4	3	4
	SW2401	148~170	7	3	5	5	5	4
	SW2456	189~211	5	3	4	5	3	5
	SW2515	90~108	9	2	7	6	5	2
	SW316	133~159	9	3	5	4	4	3
	SWR1526	114~146	5	1	4	3	4	5
	SWR1849	115~160	8	3	5	3	4	4
	SWR1941	202~222	9	2	4	5	6	4

K: Korea native pig, B: Berkshire, L: Landrace, Y: Yorkshire, D: Duroc

Alleles of MS marker from Genotyper Software were organized individual and group by analyzing using Version 3.0 program (version 3.0, The University of Edinburgh). The Heterozygosity of entire population, allele frequency and number of allele at each locus and at breed group were calculated. Also, it showed up variety of allele in marker about each breed through calculated the value of expected heterozygosity and observed heterozygosity about 5 swine breed.

RESULTS AND DISCUSSION

Specific allele appearing to comparing different species-specific alleles can be used as a measure of genetic distinction within species and between species, therefore, we were calculated the number of allele of locus and breed group about each MS marker (Table 2).

The first set consists of 11 MS marker comes out 73 alleles. Especially, SW1920 have the highest of 10 alleles and SW403, S0227 and SWR414 has the smallest of 4 alleles. The second set consists of 12 MS marker comes out 80 alleles. Among them SW2515, SW316 and SWR1941 have the highest of 9 alleles and lowest SW-1041 and SW1377 emerged as having 4 alleles. As a re-

Table 3. Expected and observed heterozygosities and PIC value at 23 microsatellite in 5 swine breed

Locus	Population														
	Korean native pig			Berkshire			Landrace			Yorkshire			Duroc		
	Ex H	Ob H	PIC	Ex H	Ob H	PIC	Ex H	Ob H	PIC	Ex H	Ob H	PIC	Ex H	Ob H	PIC
SW403	0.262	0.3	0.222	0.511	0.316	0.397	0.582	0.368	0.473	0.65	0.632	0.571	0.462	0.263	0.349
S0226	0.699	0.7	0.621	0.642	0.579	0.568	0.617	0.526	0.542	0.397	0.421	0.35	0.514	0.579	0.375
SW210	0.645	0.7	0.555	0.804	0.789	0.751	0.707	0.789	0.645	0.496	0.368	0.389	0.364	0.316	0.327
SW2410	0	0	0	0	0	0	0.289	0.158	0.267	0.59	0.526	0.511	0.536	0.263	0.411
S0107	0.437	0.316	0.354	0.671	0.474	0.594	0.651	0.333	0.565	0.788	0.895	0.728	0.323	0.316	0.288
S0227	0.185	0.2	0.164	0.309	0.263	0.255	0.193	0.105	0.171	0	0	0	0.104	0.053	0.099
SWR414	0	0	0	0.323	0.263	0.288	0.193	0.211	0.171	0.102	0.105	0.095	0.4	0.474	0.356
S0068	0.56	0.444	0.481	0.818	0.789	0.765	0.543	0.4	0.496	0.16	0.167	0.149	0.79	0.556	0.735
SW1920	0.383	0.35	0.343	0.477	0.474	0.432	0.861	0.789	0.817	0.791	0.474	0.733	0.832	0.526	0.783
SWR2516	0.501	0.55	0.369	0.643	0.632	0.562	0.616	0.579	0.554	0.639	0.526	0.548	0.422	0.474	0.327
S0101	0.185	0.2	0.164	0.368	0.368	0.336	0.661	0.632	0.576	0.512	0.579	0.467	0.391	0.368	0.338
S0018	0.097	0.1	0.09	0.546	0.211	0.474	0.579	0.263	0.51	0.626	0.368	0.54	0.694	0.556	0.612
SW1041	0.574	0.65	0.499	0.491	0.684	0.364	0.501	0.526	0.369	0.468	0.421	0.415	0.508	0.684	0.372
SW1377	0.504	0.5	0.441	0.563	0.579	0.445	0.371	0.474	0.296	0.053	0.053	0.05	0.512	0.526	0.374
SW1557	0.185	0.2	0.164	0.599	0.421	0.496	0.643	0.474	0.562	0.248	0.211	0.234	0.677	0.316	0.597
SW1989	0.344	0.4	0.303	0.61	0.579	0.511	0.73	0.789	0.662	0.465	0.368	0.409	0.599	0.579	0.513
SW2401	0.681	0.6	0.59	0.741	0.632	0.68	0.573	0.474	0.523	0.775	0.842	0.714	0.246	0.211	0.23
SW2456	0.44	0.25	0.38	0.627	0.316	0.534	0.616	0.316	0.53	0.671	0.263	0.58	0.762	0	0.7
SW2515	0.224	0.25	0.195	0.669	0.526	0.609	0.706	0.579	0.632	0.657	0.895	0.604	0.508	0.684	0.372
SW316	0.617	0.6	0.516	0.73	0.579	0.66	0.677	0.421	0.6	0.755	0.789	0.687	0.586	0.368	0.504
SWR1526	0	0	0	0.596	0.526	0.539	0.665	0.722	0.571	0.613	0.263	0.549	0.7	0.444	0.643
SWR1849	0.645	0.7	0.555	0.72	0.737	0.646	0.585	0.579	0.474	0.599	0.632	0.513	0.57	0.474	0.469
SWR1941	0.45	0.45	0.342	0.529	0.632	0.469	0.603	0.737	0.52	0.748	0.895	0.696	0.653	0.789	0.584

Ex H: Expected Heterozygosity, Ob H: Objectived Heterozygosity, PIC: polymorphic information content.

sult, total 153 alleles were genotyped to determine. Each of these breed, heterozygosity by various alleles also derived the value of a relatively wide range, but are distributed for each set.

Many studies carried out in cattle and pig, were known that discrimination of the breed over 96% shown by marker of a similar level (Fan *et al*., 2005; Oh *et al*., 2008).

Also, the old breed structure, with differentiated varieties locally distributed, has been replaced by a pyramidal structure based on crossbreeding with Duroc, and a strong dependence on a small number of breeding nuclei supplying purebred all the production tier.

In these circumstances, some ancestral varieties have disappeared, others are endangered or blended, necessitating a new design for programmes of conservation of these genetic resources (Fabuel E, 2004).

So allele number and heterozygosity will improve discrimination of each set because of various distribution of each set. Concretely, comparison using the frequency of allele expression by genetic marker of analysis target was able to detect specificity of breed group. When compared with graph of allele frequency of MS marker having relatively many alleles, it increased confidence of genetic discrimination because they represent significant differences (Fig. 1). At representative allele at each set, alleles of 5 swine breed at each locus had many difference, therefore specificity of breeding was decided easily by combination of various alleles. The observed and expected heterozygosity, and Polymorphic

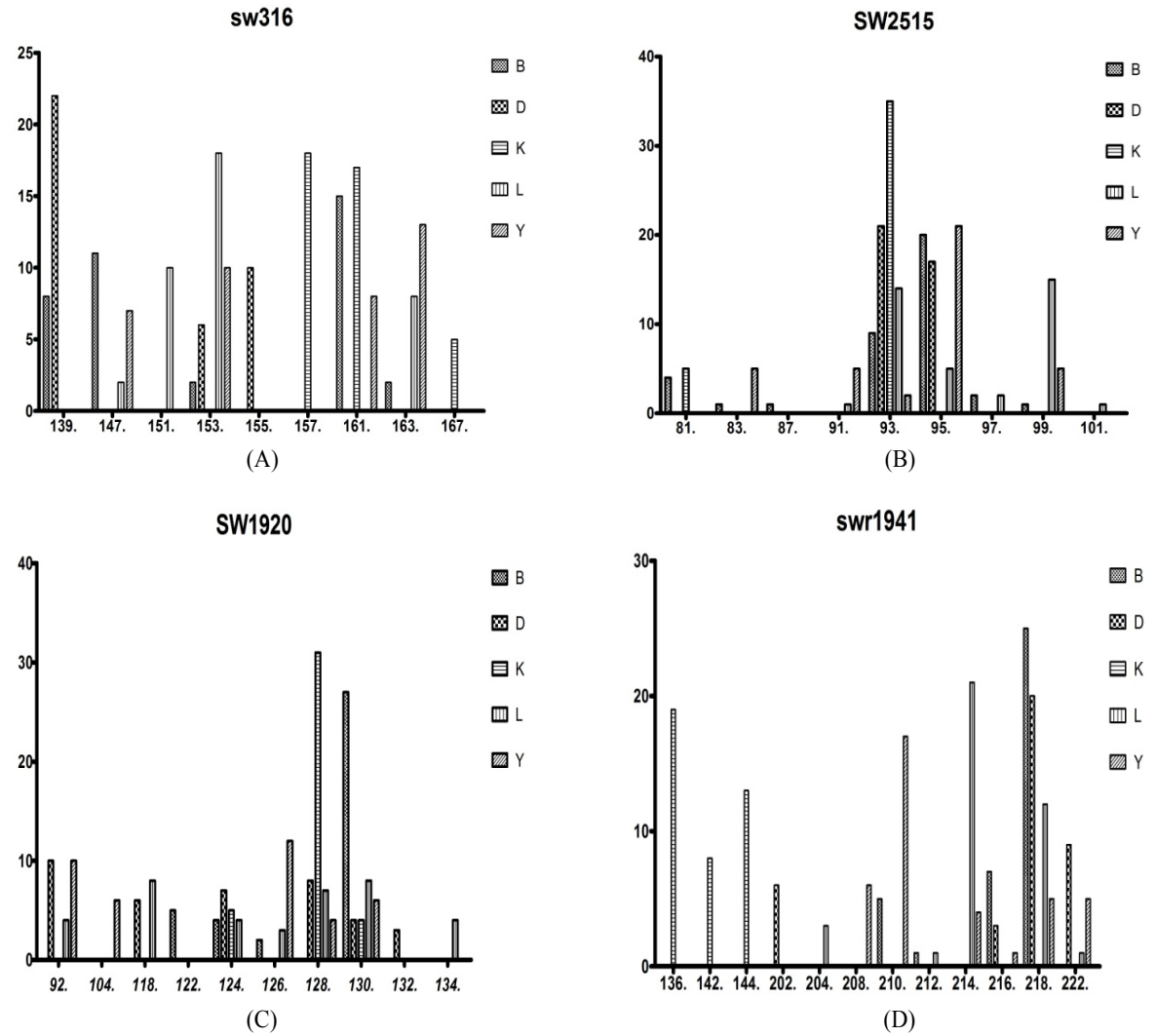

Fig. 1. Allele frequency distribution of microsatellite markers (MS marker) in 5 swine breed. K: Korea native pig, B: Berkshire, L: Landrace, Y: Yorkshire, D: Duroc.

Informative Content (PIC) were calculated to determine the genetic diversity, the results are shown in Table 3. Observed and expected heterozygosity had various values from the minimum to the maximum, except only one allele and showed relatively high value in case of PIC. In view of these results, the selected MS marker set can be understood as markers for analysis using usefulness of individual identification based on breed specificity within each group.

Based on these results, through a multiplex PCR technique using a combination of two set types of MS marker, we can improve breed-specific marker by faster, more accurate and cost-effective way by analyzing of multiple alleles showed in swine breed an can be used to parentage diagnosis and individual identification, in addition can be used as traits associated biomarker. Therefore, MS marker having very high value in the field of molecule breeding used to prevent the extinction due to inbreeding, as well as, was a technology using improvement by selecting a specific trait (Kaul *et al*., 2001). In addition, multiplex PCR technique using various MS marker at a time than a single PCR by conventional methods to confirm only one MS marker can take advantage faster, more accurate and cost-effective way for analysis charge at the genetic paternity diagnosis and individual identification (Lim *et al*., 2009).

To date, the demerits of studbook represent only documents were to break through providing a scientific evidence of swine registration system and supplementary the history tracking system, and thereby can build up to based on integrated management system at the national level of swine.

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