The Thai Journal of Veterinary Medicine

Volume 39
Issue 3 *September, 2009*

Article 8

9-1-2009

Efficacy of Microsatellite Markers in Parentage Control in Swine

Wanwisa Yaemmeeklin

Jutarat Jirasupphachok

Weerapong Koykul

Duangsmorn Suwattana

Original Article

Efficacy of Microsatellite Markers in Parentage Control in Swine

Wanwisa Yaemmeeklin[1] Jutarat Jirasupphachok[1] Weerapong Koykul[2]
Duangsmorn Suwattana[1*]

Abstract

The efficacy of microsatellite markers was evaluated in order to use in parentage control in swine. Genomic DNA from 80 samples were extracted, and amplified using 16 microsatellite markers (D00768, KVL9000, NLRIP0001, S0663, S0710, S0719, S0766, SJ859, SJ923, SJ924, SJ925, SJ926, SJ927, SJ929, X53085 and X63893) in each single polymerase chain reaction (PCR). The PCR products were analyzed using agarose and polyacrylamide gel electrophoresis (PAGE). The result showed that 15 microsatellite loci could be amplified except SJ925. Seven suitable microsatellite markers were selected for parentage control, including D00768, KVL9000, NLRIP0001, S0663, S0710, S0719, and S0766. Allelic numbers of the selected markers varied from 4 to 8. The values of observed and expected heterozygosities ranged from 0.3250 to 1.0000 and from 0.5456 to 0.8302, respectively. The polymorphic information content (PIC) was 0.5179-0.8106 and the combined exclusion probability (CEP) was 0.9946 (99.46%). The results demonstrated that the efficacy of 7 microsatellite loci was high and they can be used as a powerful tool for parentage control in swine in Thailand.

Keywords : microsatellite marker, parentage control, swine

[1]Department of Animal Husbandry [2]Department of Veterinary Anatomy, Faculty of Veterinary Science
Chulalongkorn University, Pathumwan, Bangkok 10330 Thailand.
[*]Corresponding author E-mail: duangsmorn.s@chula.ac.th

Thai J. Vet. Med., 2009. 39(3): 259-265

บทคัดย่อ

ประสิทธิภาพของเครื่องหมายพันธุกรรมไมโครแซทเทลไลท์ในการหาความสัมพันธ์พ่อ แม่ ลูกในสุกร

วันวิสาข์ แย้มมีกลิ่น[1] จุฑารัตน์ จิระศุภโชค[1] วีระพงศ์ โกยกุล[2] ดวงสมร สุวัฑฒน[1*]

การศึกษามีวัตถุประสงค์เพื่อประเมินประสิทธิภาพของเครื่องหมายพันธุกรรมไมโครแซทเทลไลท์ในการหาความสัมพันธ์พ่อ แม่ ลูก จากตัวอย่างดีเอ็นเอของสุกรจำนวน 80 ตัวอย่าง โดยใช้เครื่องหมายพันธุกรรมไมโครแซทเทลไลท์จำนวน 16 ตำแหน่ง ได้แก่ D00768, KVL9000, NLRIP0001, S0663, S0710, S0719, S0766, SJ859, SJ923, SJ924, SJ925, SJ926, SJ927, SJ929, X53085 และ X63893 ทำการเพิ่มจำนวนชิ้นส่วนดีเอ็นเอด้วยวิธีโพลิเมอเรสเชนรีแอคชัน (PCR) จากนั้นวิเคราะห์ผลผลิต PCR ด้วยวิธีอกาโรสเจล และโพลิอคริลาไมด์เจล อิเล็กโทรฟอเรซิส (PAGE) ผลการศึกษาพบว่า สามารถเพิ่มจำนวนชิ้นส่วนดีเอ็นเอได้จำนวน 15 ตำแหน่ง ยกเว้นตำแหน่ง SJ925 ทำการคัดเลือกเครื่องหมายพันธุกรรมไมโครแซทเทลไลท์ที่เหมาะสมในการศึกษาครั้งนี้ได้ทั้งสิ้นจำนวน 7 ตำแหน่ง ได้แก่ D00768, KVL9000, NLRIP0001, S0663, S0710, S0719 และ S0766 ซึ่งตรวจพบจำนวนอัลลีลอยู่ระหว่าง 4-8 อัลลีล นอกจากนี้จากการคำนวณค่าเฮทเทอโรไซโกซิติจากการสังเกต (Hobs) และจากทฤษฎี (Hexp) พบว่ามีค่า 0.3250-1.0000 และ 0.5456-0.8302 ตามลำดับ ค่าโพลีมอร์ฟิคอินฟอร์มเมชั่นคอนเท้นท์ (PIC) มีค่า 0.5179-0.8106 และ ค่าความแม่นยำในการวิเคราะห์ผลเมื่อนำเครื่องหมายไปใช้งานร่วมกัน (CEP) มีค่าเท่ากับร้อยละ 99.46 ในการศึกษาครั้งนี้แสดงให้เห็นว่าเครื่องหมายพันธุกรรมไมโครแซทเทลไลท์ทั้ง 7 ตำแหน่ง มีประสิทธิภาพในการใช้งานสูง และสามารถใช้เป็นเครื่องมือสำหรับตรวจสอบความสัมพันธ์พ่อ แม่ ลูกของสุกรในประเทศไทยได้ดี

คำสำคัญ : เครื่องหมายพันธุกรรมไมโครแซทเทลไลท์ ความสัมพันธ์พ่อ-แม่-ลูก สุกร

[1]ภาควิชาสัตวบาล [2]ภาควิชากายวิภาคศาสตร์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปทุมวัน กรุงเทพฯ 10330
[*]ผู้รับผิดชอบบทความ E-mail: duangsmorn.s@chula.ac.th

Introduction

Swine production in Thailand has been continuously developed by the import of high quality breeding stock and semen from abroad. In addition, breeding and selection have been applied to increase the domestic production. Therefore, genetic parameters in terms of pedigree and progeny records, play an important role in swine breeding (Weller et al., 2004). It is estimated that 20% discrepancies in progeny records could lead to a significant decrease in genetic progress calculated by Best Linear Unbiased Prediction (BLUP) (Banos et al., 2001). Based on this, incomplete records on mating, transfer of piglets, and use of multiple sires, could hamper the progress in swine breeding. At present, advances in molecular genetic techniques allow the possibility of testing on genetic polymorphism, phylogenetic studies and parentage identification. Microsatellite DNAs, which are simple nucleotide repeats, have been widely used as markers in parentage control due to their high degree of polymorphism. The number and pattern of microsatellite marker alleles vary in each animal, animals in each population and animals in different populations (Tóth et al., 2000). In addition, microsatellite DNAs can be multiplied in numbers using polymerase chain reaction (PCR) in which a marker of 200-600 bp with tri- or tetranucleotide repeats, has been shown to be the most suitable (Lai and Sunny, 2003). The global objectives of this study were to evaluate the efficacy of microsatellite markers in order to use in parentage control and identification of swine in Thailand.

Materials and Methods

Animals: Blood and semen samples were collected from two groups of swine: the non- and the genetically related groups. The non-genetically related group consisted of 26 animals from two farms in Ratchaburi province and 14 animals from a farm in Chai-nart province. Animals in this group served to provide information on the polymorphism and diversity of each microsatellite marker. The genetically related group comprised 40 animals from farms in Nakornpathom province and Chai-nart province. Swine in this group provided results from the comparison of progeny records and parentage identification using microsatellite markers.

Microsatellite markers: Sixteen microsatellite loci were selected from the database of the National Center for Biotechnology Information (NCBI, 2008) by choosing tri- or tetranucleotide repeats with the number of 100 bp or more, as shown in Table 1.

DNA analyses: DNA was isolated from collected samples using QIAamp® DNA Mini Kit, according to the manufacturerís instruction. Isolated DNAs were assessed and quantified using agarose gel electrophoresis before subjected to polymerase chain reaction (PCR) using each primer of 16 selected microsatellite markers. The annealing temperatures for PCR ranged from 56 to 60°C. PCR products were then run on agarose gel electrophoresis to assess their quality and quantity, before subjected to polyacrylamide gel electrophoresis (PAGE). Allele sizes of each marker shown on PAGE were recorded.

Statistical analyses: Allele frequency, observed heterozygosity (Hobs) and expected heterozygosity (H_{exp}) were calculated according to the methods described by Nei (1978). Efficacy of microsatellite markers were evaluated using Polymorphic Information Content (PIC) (Bolstein et al., 1980), Exclusion Probability (EP) (Wang, 2007) and Combined Exclusion Probability (CEP) (Jamieson and Taylor, 1997).

Results

PCR Products: Amplifications of microsatellite DNAs using polymerase chain reactions resulted in PCR products of all markers except SJ925. Analyses of DNA fragments using polyacrylamide gel electrophoresis (PAGE) revealed that only 7 markers demonstrated polymorphism suitable for using in parentage control. These markers included D00768, KVL9000, NLRIP0001, S0663, S0710, S0719 and S0766.

Microsatellite Polymorphism: The number, distribution pattern and frequency of alleles of 7 polymorphic markers and the values of observed heterozygosity, expected heterozygosity and Polymorphic Information Content, are shown in Table 2. It was found that each of 7 loci expressed different patterns of allele frequency and PIC values with a marker S0663 expressing the highest number of alleles and PIC value (Figure 1 and Table 2). The tested markers can be categorized into two groups: those of almost equal proportions for each frequency (D00768, KVL9000 and S0663), and those with varied allele proportions (NLRIP0001, S0710, S0719 and S0766), as shown in Figure 2.

Efficacy of Microsatellite Markers: Estimation of Exclusion Probability (EP) and Combined Exclusion Probability (CEP) for 7 microsatellite loci revealed that S0663 expressed the highest EP value (0.6692) while S0710 had the lowest (0.3443). Combined Exclusion Probability values from the combination of 2 to 7 markers ranged from 0.8716 to 0.9946. Therefore, using all 7 markers in parentage identification could yield the accuracy to the degree of 99.46%. Results of parentage identification using microsatellite markers corresponded to the progeny record of the genetically related group. Exclusion Probability and CEP values from 80 samples are shown in Table 3 and the relationship between EP values and the number of markers used is shown in Figure 3.

Table 1 Microsatellite markers

Locus name	Primer sequences (5'-3')	Core sequences	Approximated Size (bp)	Accession no.
D00768	F: GACACAGTGGATGGCATTTG	$(CTTT)_n$	340	D00768
	R: ACATCCCTAAGGTCGTGGC			
KVL9000	F: TGCAAAGTTTGGGACATCAG	$(GATA)_n$	260	EU010405
	R: AGGTGCTGAGGATACAGTGG			
NLRIP0001	F: GATCTCAGCTTCAATACCTCC	$(TTTC)_n$	340	AY740518
	R: GATCCTGTATTGCTGTGGCTG			
S0663	F: TGGTTCGGGAACATAGGAAAAG	$(ATAG)_n$	240	AJ544213
	R: AGCTGGGTCCTCCATATGCTG			
S0710	F: CTCAGCACCTTACAAACC	$(TAAA)_n$	330	AY253998
	R: TCCCAAACCAATCCACAC			
S0719	F: TCTCCAAGTCCAGGAACTTGC	$(GAAA)_n$	600	AY451240
	R: TCGCCATACTCTTCTAATGGC			
S0766	F: GTGTAGATATGTGTCTGTACA	$(GAAA)_n$	620	AY731063
	R: AGACCTCCTATTAGAGGTGGA			
SJ859	F: TCAAGAGAAAAGGACAAAATC	$(TTTG)_n$	330	AB248496
	R: ATGAAGAGGTGGAGACTGTG			
SJ923	F: CCAAGAAATAGCAACAACAA	$(CAA)_n$	200	AB248491
	R: AGATGATTTGGTTTGGTCTTA			
SJ924	F: GATTTGTTTCCGCTGAGCCA	$(AAC)_n$	230	AB248492
	R: TGGGCTCACAGGCACAGTATC			
SJ925	F: CACAAAGGAGGAGGCTGGAAT	$(TTG)_n$	380	AB248490
	R: TTGCTGTGGTCTGGCGTAGG			
SJ926	F: CTACCACTGAGCCACAAGAG	$(TTA)_n$	240	AB248494
	R: TGGTGTAGATTTCAGATGCTG			
SJ927	F: CTCAGTGTGGCATTCAGGTC	$(TTG)_n$	270	AB248493
	R: TGACCTACACCACAGCTCATG			
SJ929	F: ATGACCCAGGAACAAGGATAG	$(TTTG)_n$	330	AB248487
	R: TCAAAGAAATGGGGAAACAG			
X53085	F: TGTTCAGTGGGTTAAGGATCG	$(AGGA)_n$	400	X53085
	R: TTCCCTACACCCTGCCTTC			
X63893	F: GGGTCAGACCGACACCAC	$(GCC)_n$	120	X63893
	R: GGTCTTGCTGTTTCCGAGAC			

Sources: National Center for Biotechnology Information (NCBI)

Table 2 The number, distribution pattern and frequency of alleles; Observed and expected heterozygosities and Polymorphic Information Contents of 7 microsatellite markers.

Markers	S0663	D00768	KVL9000	S0719	NLRIP0001	S0766	S0710
No. of alleles	8	6	7	5	6	4	6
Alleles	frequency (n=80)						
A	0.2938	0.0812	0.1750	0.1563	0.4313	0.3625	0.6500
B	0.0562	0.0750	0.0750	0.3438	0.1938	0.0937	0.0440
C	0.0562	0.2063	0.0937	0.1437	0.2062	0.4688	0.0810
D	0.1187	0.2625	0.0563	0.0437	0.0250	0.0750	0.0875
E	0.1063	0.1625	0.3375	0.3125	0.0750		0.1250
F	0.1000	0.2125	0.2125		0.0687		0.0125
G	0.1875		0.0500				
H	0.0813						
H_{Obs}	0.6375	0.7125	0.8875	0.3250	0.6750	1.0000	0.6250
H_{exp}	0.8302	0.8048	0.7902	0.7372	0.7229	0.6345	0.5456
χ^2	4.3101	1.0522	1.2129	25.0112	0.3512	26.5460	1.6943
PIC	0.8106	0.7760	0.7623	0.6926	0.6841	0.5665	0.5179

Table 3 Exclusion Probability (EP) and Combined Exclusion Probability (CEP) values of 7 microsatellite markers.

Markers	Exclusion probability (EP)					
	2 loci	3 loci	4 loci	5 loci	6 loci	7 loci
S0663	0.6692	0.6692	0.6692	0.6692	0.6692	0.6692
D00768	0.6117	0.6117	0.6117	0.6117	0.6117	0.6117
KVL9000		0.5992	0.5992	0.5992	0.5992	0.5992
S0719			0.5009	0.5009	0.5009	0.5009
NLRIP0001				0.4991	0.4991	0.4991
S0766					0.3647	0.3647
S0710						0.3443
CEP	0.8716	0.9485	0.9743	0.9871	0.9918	0.9946

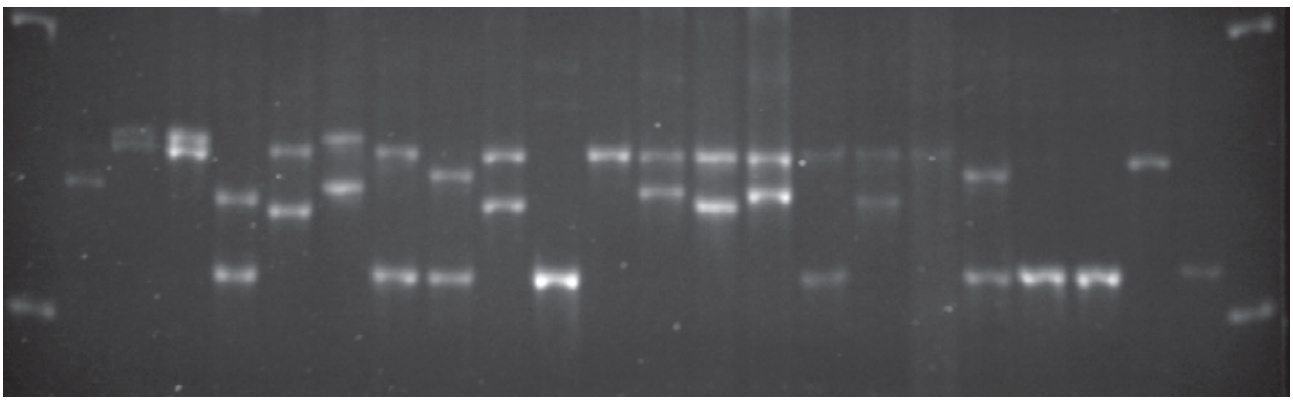

Figure 1 Distribution of alleles from marker S0663, ranging between 200-300 bp.

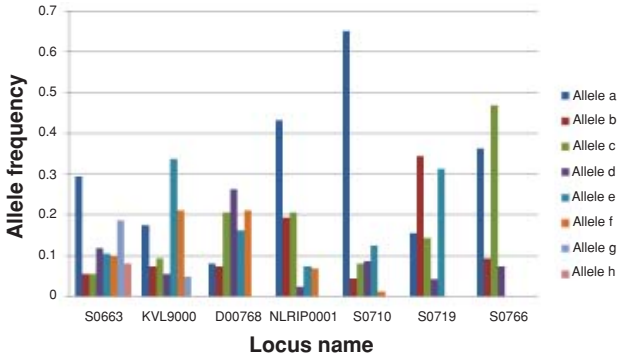

Figure 2 Distribution of alleles for each locus of 7 polymorphic markers.

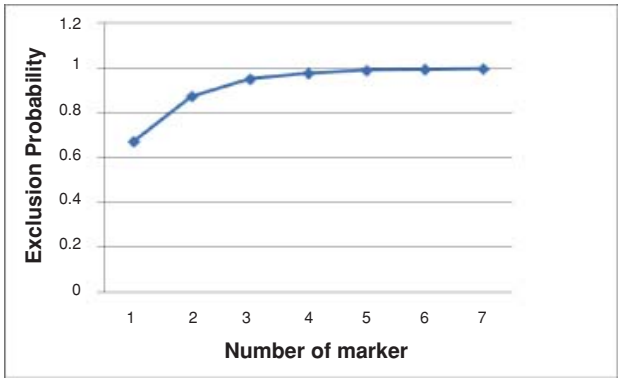

Figure 3 Relationship between EP values and the number of markers used.

Discussion

From 16 microsatellite loci tested, only 7 markers including D00768, KVL9000, NLRIP0001, S0663, S0710, S0719 and S0766, demonstrated polymorphism suitable for using in parentage identification and control. Markers D00768, KVL9000 and S0663 expressed the number of alleles and PIC values as 6, 7, 8 and 0.7760, 0.7623, 0.8106, respectively. These 3 markers indicated high efficacy since their PIC values were close to 1.000 (Jakabova et al., 2002). For markers NLRIP0001, S0710, S0719 and S0766, their numbers of alleles were 6, 6, 5 and 4 respectively and the PIC values ranged from 0.5179 to 0.6926, indicating of their moderate efficacy. However, Chen et al. (2006) reported the PIC values of S0710, S0719 and S0766 as 0.74, 0.94 and 0.86 respectively, which were somewhat different from those in our study, suggesting that efficacy of microsatellite markers could vary among different populations.

Observed and expected heterozygosities were estimated in order to assess the heterozygous state of each genotype. It was found that Hobs of S0719 was significantly lower than its Hexp, indicating that most of their genotypes were homozygous. By the contrary, the Hobs of S0766 was significantly higher than its Hexp, suggesting that a majority of their genotypes were heterozygous. In addition, Hexp and PIC values of each microsatellite locus expressed propensity of either an increase or a decrease towards the same direction. This means that Hexp values can also be used to evaluate the efficacy of markers when PIC values are not available. In the present study, S0663 appeared to be the most efficient marker (with the highest PIC and Hexp values) whereas S0710 was the least efficient, with the lowest PIC and Hexp values. Since PIC and Hexp values were estimated from the number and frequency of alleles, these two parameters should be considered for the evaluation of efficacy for each marker (Radko and Slota, 2007).

Exclusion Probability (EP) and Combine Exclusion Probability (CEP) values were used to determine the efficacy of using microsatellite markers in parentage identification. It is normally found that the CEP value increases according to the number of markers used, in combination. In this study, when all 7 microsatellite loci were used, the CEP value was 0.9946, indicating that the accuracy of using these markers combined, would be 99.46%. In general, CEP values could be increased when more markers were utilized although it was not true in all cases, depending on the quality of markers used. Putnova et al. (2003) applied 10 microsatellite loci in parentage identification in swine and found that the CEP value was as high as 0.9994 (99.94%) whereas Rohrer et al. (2007) utilized 10 markers and the CEP value was 0.9904 (99.04%), which was lower than that in our study (using 7 markers). However, when the number of markers used was put up to 15, the CEP value could be near 100% as in one study in swine (Nechtelberger et al., 2001). Therefore, the number of markers to be used should be considered

along with other factors such as cost and time, in order to reach the accepted accuracy.

In conclusion, for all 80 swine used in this study, we were able to utilize 7 microsatellite markers in order to identify and differentiate each animal with 99.46% accuracy, indicating that there was only 0.54% chance that two animals would be identically similar and identified as one. Therefore, all seven markers selected in this study are suitable for using in swine parentage control in Thailand since the results corresponded to the pedigree record of the genetically related group. However, 2-3 microsatellite loci with PIC values between 0.8-0.9, can be added in order to increase the accuracy to 99.99%.

Acknowledgments

The authors would like to thank Dr. Teewakorn Sirichokchatchawan for kindly providing samples used in this study, and the Innovation Center for Veterinary Science, Chulalongkorn University for facilitating all necessary equipments. This work was supported by grants from the Graduate School and Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand.

References

Banos, G., Wiggans, G.R. and Powell, R.L. 2001. Impact of paternity errors in cow identification on genetic evaluation and international comparisons. J. Dairy Sci. 84: 2523-2529.

Botstein, D., White, R.L., Skolnick, M. and Davis, R.W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32: 314-331.

Chen, K., Knorr, C., Bornemann-Kolatzki, K., Huang, L., Rohrer, G.A. and Brenig, B. 2006. Characterization of the PGK2 associated microsatellite S0719 on SSC7 suitable for parentage and QTL diagnosis. Anim. Biotech. 17: 43-49.

Jakabova, D., Trandzik, J., Chrastina, J., Hudecova, L., Zetochova, E., Bulla, J., Bugarsky, A., Jakab, F. and Kozlik, P. 2002. Effectiveness of six highly polymorphic microsatellite markers in resolving paternity cases in Thoroughbred horses in Slovakia. Czech J. Anim. Sci. 47: 497-501.

Jamieson, A. and Taylor, S.S. 1997. Comparisons of three probability formulae for parentage exclusion. Anim. Genet. 28: 397-400.

Lai, Y. and Sunny, F. 2003. The relationship between microsatellite slippage mutation rate and the number of repeat units. Mol. Biol. Evol. 20: 2123-2131.

NCBI. 2008. "CoreNucleotide" [Online]. Available: http://www.ncbi.nlm.nih.gov/.

Nechtelberger, D., Kaltwasser, C., Stur, I., Meyer, J-N., Brem, G., Mueller, M. and Mueller, S. 2001. DNA microsatellite analysis for parentage control in Austrian pigs. Anim. Biotech. 12: 141-144.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.

Putnova, L., Knoll, A., Dvorak, V. and Dvorak, J. 2003. A novel porcine microsatellite panel for the identification of individuals and parentage control in the Czech Republic. Czech J. Anim. Sci. 48: 307-314.

Radko, A. and Slota, E. 2007. Polymorphism of 11 microsatellite DNA sequences used for parentage control in Holstein-Friesian bulls of black and white variety in Poland. Ann. Anim. Sci. 2: 189-196.

Rohrer,G.A., Freking, B.A. and Nonneman, D. 2007. Single nucleotide polymorphisms for pig identification and parentage exclusion. Anim. Genet. 38: 253-258.

T?th, G., G?spari, Z. and Jurka, J. 2000. Microsatellites in different eukaryotic genomes: survey and analysis. Genome Res. 10: 967-981.

Wang, J. 2007. Parentage and sibship exclusions: higher statistical power with more family members. Heredity 99: 205-217.

Weller, J.L., Feldmesser, E., Golik, M. Tager-Cohen I., Domochovsky, R., Alus, O. Ezra, E. and Ron, M. 2004. Factors affecting incorrect paternity assignment in the Israeli Holstein population. J. Dairy Sci. 87: 2627-2640.