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Characterization of swine leukocyte antigen polymorphism by sequence-based and PCR-SSP methods in Meishan pigs

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Abstract Resource herds of swine leukocyte antigen (SLA)-characterized pigs are an important tool for the study of immune responses, disease resistance, and production traits. They are also valuable large animal models for biomedical research, such as transplantation. The Meishan breed of pig is an economically significant breed that is available at several research institutions in the United States. We have characterized the SLA polymorphism of the breeding stock in the herd maintained at the University of Illinois and developed a simple assay to SLA type individuals within that herd. We have used a reverse transcription-polymerase chain reaction (RT-PCR)-based SLA typing method to clone and DNA sequence 19 SLA alleles at three SLA class Ia (*SLA-1*, *SLA-2*, and *SLA-3*) and two SLA class II (*SLA-DRB1* and *SLA-DQB1*) loci. Based on this sequence information, a rapid SLA typing assay was developed to discriminate each allele using PCR with sequence-specific primers (PCR-SSP). Using this method, we were able to characterize the entire Meishan breeding stock and identify four SLA haplotypes present in the herd. The combination of SLA typing by cloning and DNA sequencing with PCR-SSP is therefore a valuable tool for the characterization of SLA alleles and haplotypes in resource herds of pigs.

Keywords Major histocompatibility complex · Meishan · Pig · Sequence-specific primer · Swine leukocyte antigen

Abbreviations

Hp	haplotype
ISAG	International Society for Animal Genetics
MHC	major histocompatibility complex
PCR	polymerase chain reaction
RFLP	restriction length polymorphism
RT	reverse transcription
SLA	swine leukocyte antigen
SSP	sequence-specific primer

The nucleotide sequence data reported in this article have been submitted to the DDBJ/EMBL/GenBank databases under the accession numbers AF464049, AF464050, AF464051, AF464059, AY459297, AY459299, AY459300, AY459301, AY459306, DQ303218, DQ303219, DQ303220, DQ303221, DQ303222, DQ303223, DQ303224, DQ303225, DQ303226, DQ303227, DQ303228, DQ303229, and DQ303230.

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Introduction

The porcine major histocompatibility complex (MHC), which codes for the swine leukocyte antigens (SLA), has

been mapped to pig chromosome 7 near the centromere (Geffrotin et al. 1984; Smith et al. 1995). The SLA genes encode for extremely polymorphic class I and class II glycoproteins that function mainly in presenting self and nonself peptides on the surface of cells to T lymphocytes, and therefore play a vital role in the development and control of the immune system.

SLA alleles and haplotypes have been repeatedly associated with difference in swine immune response to various infectious agents (Lunney and Murrell 1988; Mallard et al. 1989) and vaccinations (Lumsden et al. 1993; Rothschild et al. 1984). SLA polymorphism has also been linked to reproductive performance and production traits (Renard and Vaiman 1989; Gautschi and Gaillard 1990). The human xenogeneic T-cell response to porcine xenografts has been shown to be SLA allele- or haplotype-specific (Xu et al. 1999, Yamada et al. 1995). Understanding the genetic background, diversity and polymorphism of the SLA alleles in pigs will therefore facilitate the study of swine immune responses for both animal health and biomedical research.

Extensive polymorphism of the SLA alleles has been well documented due to the efforts of many researchers around the world. Numerous SLA alleles and haplotypes have been characterized in many breeds of pig. A systematic nomenclature of the class I and class II SLA alleles has recently been established by the SLA Nomenclature Committee of the International Society for Animal Genetics (ISAG) (Smith et al. 2005a,b). The current allele information is now publicly available at the Immune Polymorphism Database (IPD-MHC) website at http://www.ebi.ac.uk/ipd/mhc/sla/ (Ellis et al. 2006).

The Meishan breed of pigs was introduced into the United States from China under a cooperative effort of the United States Department of Agriculture and multiple partners including the University of Illinois. It has been characterized as an extremely prolific breed with a large litter size, early sexual maturation, and high fat deposition (Legault 1985). It is also considered to be resistant to some diseases (Michaels et al. 1994; Reiner et al. 2002). The Meishan has been an economically important breed, and it has been studied extensively in regards to agricultural performance, yet its SLA genes have never been fully characterized by DNA sequencing. We have previously established a reverse transcription-polymerase chain reaction (RT-PCR)-based SLA typing method by cloning and nucleotide sequencing (Smith et al. 2005c), and rapid haplotyping assays using PCR with sequence-specific primers (PCR-SSP) for SLA typing of offspring in two experimental herds of pigs (Martens et al. 2003). In this study, we present the characterization of SLA alleles in the Meishan herd maintained at the University of Illinois and the subsequent development of a SLA genotyping assay using the PCR-SSP method.

Materials and methods

Selection of animals

Blood and semen samples were obtained from 20 purebred Meishan pigs in accordance with University of Illinois IACUC approved protocols. These animals are descendents of the original herd imported from China and represent the current breeding stock maintained at the University of Illinois Imported Swine Research Center. The original animals consisted of 21 females and 10 males. Another Meishan sample (pig MS) was generously provided by Dr. Nicholas Zavazava at the University of Iowa (also derived from the University of Illinois herd). These animals were initially SLA characterized at their SLA class Ia loci (*SLA-1*, *SLA-2*, and *SLA-3*) using PCR with low-resolution group-specific primers. Briefly, group-specific primers were designed at the polymorphic motifs that would distinguish the members of each of the allelic groups assigned by the SLA Nomenclature Committee of ISAG. Four haplotype patterns (local designations as K, L, M, and N) were deduced from the preliminary results (unpublished data). Five heterozygous pigs, 5011 (LM), 446R (KM), 1–13 (LN), 5–4 (KN), and MS (MN), representing each haplotype in at least two animals, were chosen to be further characterized by cloning and DNA sequencing.

Reverse transcription and locus-specific PCR amplification

Total RNA of the selected pigs was isolated from peripheral blood cells using the Trizol reagent (Invitrogen, Carlsbad, CA, USA). cDNA was synthesized from 1–2 µg of RNA using the ThermoScript RT-PCR system with oligo-dT primers (Invitrogen). Locus-specific primers for five loci (*SLA-1*, *SLA-2*, *SLA-3*, *SLA-DRB1*, and *SLA-DQB1*) were designed in the untranslated regions to amplify the entire coding region (Table 1). Several of these primers have been previously described (Smith et al. 2005c). The *SLA-3* allele of the M haplotype did not amplify with the usual full-length *SLA-3* locus-specific primer pair. Therefore, we used a modified primer pair based on a study by Ando et al. (2003) to amplify a partial coding sequence (partial exon 1, exon 2, and partial exon 3). Amplifications were performed with a MJ Research PTC-200 thermal cycler (Bio-Rad, Waltham, MA, USA) in 20 µl reaction volume containing cDNA sample, 1 U of *PfuUltra* Hotstart High Fidelity DNA polymerase with the supplied 1X reaction buffer (Stratagene, La Jolla, CA, USA), 0.2 mM of each dNTP (Promega, Madison, WI, USA) and 1.0 pmol/µl of each locus-specific primer pair (2.0 pmol/µl for the full-length *SLA-3* primers; 1.6 pmol/µl for the partial *SLA-3* primers). The cycling conditions included an initial incubation of 2 min at 95°C followed by 35 cycles of 95°C for 30 s, 50°C

Table 1 Locus-specific primer sets used for amplification of the SLA class I and class II genes in Meishan pigs

Locus	Primer ID	Position[a]	Forward and reverse primer sequence (5′→3′)	Estimated product size[b]
SLA-1	SLA1/3f#92	−2	CCAGACTCCGAGGCTGAGGAT	1,512 bp
	SLA1r#119	+1,465	TTCTCAATCCTTCCATTTATTTCCTC	
SLA-2	SLA2f#55[c]	−5	CCACAGAATCTCCGCAGATTCC	1,232 bp
	SLA2r#56	+1,183	CCGACACAGACACATTCAAATGCT	
SLA-3[d]	SLA1/3f#92	−2	CCAGACTCCGAGGCTGAGGAT	1,494 bp
	SLA3r#121	+1,450	TAGGCTCTTTTCCCTTGGTTAGG	
SLA-3[e]	SLA3f#115	+54	GRCCCTGGCCCTGACTGGK	533 bp
	SLA3r#116	+550	GGAGCCACWCCACACACGC	
SLA-DRB1	DRBf#89[c]	−3	TGTCCTCTCCTGTTCTCCA	907 bp
	DRBr#90[c]	+868	AGGACGCAGAGCATAGCAG	
SLA-DQB1	DQBf#80[c]	−13	TGACTACCATTACTTCTTCGT	1,103 bp
	DQBr#81[c]	+1,051	TCTTGCACAGTCTGTTGAGG	

[a] Referred to the 3′ end of the primers and relative to the start codon based on the H01 haplotype (GenBank accession number AJ251829 and AJ131112)

[b] Calculated based on the H01 haplotype. May vary slightly due to polymorphism in coding sequence and/or untranslated regions

[c] Smith et al. 2005c

[d] Did not amplify with *SLA-3* of the M haplotype (Hp-18.14)

[e] Alternative primer pair modified from Ando et al. (2003) which amplify partial exon 1, exon 2, and partial exon 3

(65°C when using the partial *SLA-3* primers) for 30 s, 72°C for 3 min, and a final extension at 72°C for 30 min.

Cloning and sequencing

PCR products were electrophoresed in 1% low-melting-point agarose gels (Promega) in 1X TAE and products of predicted size were excised and digested with AgarACE (Promega). Purified PCR products were ligated into the pCR4Blunt-TOPO plasmid vector and transformed into the TOP10 One Shot chemically competent bacterial cells using the Zero Blunt TOPO PCR Cloning Kit for Sequencing (Invitrogen). Transformed bacteria were plated onto Luria–Bertani agar containing 50 μg/ml Kanamycin for clone selection. At least eight clones for each locus in each animal were initially selected. Additional clones were sequenced to resolve discrepancies if necessary. Selected clones were cultured in the CircleGrow broth (Qbiogene, Irvine, CA, USA) contained 50 μg/ml Kanamycin. Plasmids were isolated using the QIAprep Spin Miniprep Kit (Qiagen, Valencia, CA, USA). Sequencing reactions were performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), and nucleotide sequences were determined using a Genetic Analyzer 3100 (Applied Biosystems). In some cases, sequencing was performed by a custom sequencing service (SeqWright, Houston, TX, USA). Combinations of vector primers (T7 and T3), locus-specific primers (Table 1), and internal sequencing primers (Table 2) were used to determine the DNA sequence of the inserts. Several of the internal sequencing primers have been previously described (Smith et al. 2005c). Each allele was characterized by sequencing of complete coding sequence in both forward and reverse directions from at least two pigs or two PCR amplifications, with the exception of *SLA-3* allele of the M haplotype. Only a partial sequence was obtained from the *SLA-3* of the M haplotype because it failed to amplify with the usual full-length locus-specific primers.

Sequence analysis, phylogeny, and nomenclature

Nucleotide sequences were analyzed using the Wisconsin Package Version 10.0 (Genetics Computer Group, Madison, WI, USA). The complete coding sequence of each allele was assembled by overlapping forward and reverse sequence fragments. All sequences have been submitted and compared with the GenBank database. Multiple sequence alignment was created for each locus (supplementary materials). Novel alleles were subjected to analysis by the Bayesian Inference of Phylogeny (MrBayes version 3.0B4) (Huelsenbeck and Ronquist 2001) as described previously (Smith et al. 2005a,b). Phylogenetic trees were constructed based on the polymorphism in exon 2 for class II alleles, and exon 2 and 3 for class I alleles (supplementary materials). On the basis of the phylogenetic analyses and sequence comparisons, novel alleles as well as the corresponding haplotypes were assigned official names by the ISAG SLA Nomenclature Committee.

SLA typing by PCR-SSP

Based on the sequence information obtained, sequence-specific primers were designed for the polymorphic sites to discriminate each of the alleles present in the herd (Oligo

Table 2 Internal sequencing primers

Locus	Primer ID	Position[a]	Direction	Primer sequence (5′→3′)
SLA Class Ia	SLA-1/2/3f#41	+457	Forward	GAACGAGGACCTGCGCT
SLA Class Ia	SLA-1/2/3r#42	+983	Forward	CTGGAGTTGTGATCTGGAG
SLA Class Ia	SLA-1/2/3r#53	+439	Reverse	CGCAGGTCCTCGTTCAG
SLA Class Ia	SLA-1/2/3r#62	+967	Reverse	CTTCCTCCAGATCACAACTCC
SLA Class Ia	SLA-1/2/3f#78	+700	Forward	GGCTTCTACCCTAAGGAGA
SLA-DRB1	DRBr#47[b]	+466	Reverse	GAACCACCTGACCTCCAC
SLA-DRB1	DRBf#48[b]	+446	Forward	CCTGGTCTGCTCTGTGAC
SLA-DQB1	DQBf#49[b]	+362	Forward	TACCAGATAGAGGAAGGCAC
SLA-DQB1	DQBf#50[b]	+741	Forward	CTGATCTTCCTCGGGCTG
SLA-DQB1	DQBr#51	+751	Reverse	CTGACTCCTGTGACGGAT
SLA-DQB1	DQBr#100[b]	+342	Reverse	TGCCTTCCTCTATCTGGTAG

[a] Referred to the 3′ end of the primers and relative to the start codon (add 9 bp for *SLA-2*)
[b] Smith et al. 2005c

version 4.05 primer design software, National Biosciences, Plymouth, MN, USA) (Table 4). Genomic DNA of each animal was extracted either from blood or semen. All PCR reactions and thermal cycling conditions were setup in 96-well format as previously described for the NIH miniature pig with minor modifications (Martens et al. 2003). In each reaction, 1X PCR Gold Buffer (Applied Biosystems), 1X Cresol Red PCR-compatible gel loading buffer (0.1 mM Cresol Red and 175 mM sucrose) and 35 ng of genomic DNA were used. Amplifications were performed either in a MJ Research PTC-200 (Bio-Rad) or a GeneAmp 2700 thermal cycler (Applied Biosystems). PCR products were electrophoresed in 2.5% agarose gels in 1X TBE at 150 V for 5 min using the Micro SSP Gel System (One Lambda, Canoga Park, CA, USA).

Results

DNA sequence-based SLA typing

Four SLA haplotypes, local designations K, L, M, and N, were identified in the Meishan pigs based on the PCR-SSP typing with SLA class I low resolution group-specific primers (unpublished data). Five heterozygous animals (pig MS, 5011, 446R, 1–13, and 5–4), representing each haplotype in at least two animals, were selected to be further characterized by cloning and nucleotide sequencing at five SLA loci (*SLA-1*, *SLA-2*, *SLA-3*, *SLA-DRB1*, and *SLA-DQB1*).

A total of 19 alleles were identified. Their complete coding sequences (partial coding sequence for *SLA-3* of the M haplotype) and protein sequences have been deduced and submitted to GenBank (Table 3). Comparisons with other published sequences in the GenBank database indicated 10 of the alleles were novel. These were subjected to phylogenetic analyses (supplemental materials), which were used for group and name assignments by the ISAG SLA Nomenclature Committee. Official ISAG allele designations are shown in Table 3.

One novel *SLA-DQB1* allele of the L haplotype was nearly identical to the *SLA-DQB1**0401 found in the NIH miniature pigs and the Large White pigs (GenBank accession numbers AF464026 and AY247779, respectively), except at position +270. To exclude the possibility of this synonymous polymorphism being a PCR artifact or DNA sequencing error, it was confirmed by designing a PCR-SSP primer directed at this site (data not shown). This allele was therefore assigned as a confirmed allele (*SLA-DQB1** 040102) (The use of the fifth and sixth digits in the allele name indicates that the protein sequence is identical to *SLA-DQB1**040101). The *SLA-3* allele of the N haplotype was found to be identical to *SLA-3**06an03 found in the Clawn miniature swine (GenBank accession number AB105389) and was assigned with a permanent name as *SLA-3**0602 (new alleles are given temporary alpha-numeric names until they are confirmed in another laboratory or in another breed).

Although at least eight clones for each locus in each animal were screened, there were occasions that only one allele was detected when two were actually present in the animal, particularly when the locus-specific primer pair preferentially amplifies one of the two alleles. We were able to identify two allelic sequences for each locus except for *SLA-1*, *SLA-3*, and the class II loci of pig 446R. Only one *SLA-1* allele was found in pig 446R. PCR-SSP results indicated that this was due to the K and M haplotypes sharing the *SLA-1**0401 allele rather than missing a second allele. Pig 446R also had only one allele for the *SLA-DRB1* and *SLA-DQB1* loci and PCR-SSP results indicated that this was due to the K and M haplotypes sharing these alleles as well. Four of the animals had more than two *SLA-1* alleles. This suggests that two of the haplotypes (N and L) have a duplicated *SLA-1* locus, which has also been found in several other haplotypes from other pig breeds (Smith et al. 2005a).

Table 3 The SLA class I and class II alleles identified in five Meishan pigs by RT-PCR-based cloning and nucleotide sequencing methods and their GenBank accession numbers

Pig/Locus	*SLA-1*	GenBank number	*SLA-3*	GenBank number	*SLA-2*	GenBank number	*SLA-DRB1*	GenBank number	*SLA-DQB1*	GenBank number
MS	0401	AY459306	03cs01[a]	DQ303226	06me01	AF464049	0901	AF464050	0801	AY459301
	w08ms05	AY459299	0602[a]	DQ303227	w09sn01	AF464059	0401	AF464051	0201	AY459300
	w13ms21	AY459297								
5011	0401	AY459306	0101[b]	DQ303224	06me01	AF464049	0901	AF464050	0801	AY459301
	w10cs01	DQ303230			w11cs01	DQ303222	cs01	DQ303220	040102	DQ303218
	cs02	DQ303229								
446R	0401	DQ303228	0401[b]	DQ303225	06me01	AF464049	0901	DQ303221	0801	DQ303219
					0401	DQ303223				
1–13	w08ms05	AY459299	0101[b]	DQ303224	w09sn01	AF464059	0401	AF464051	0201	AY459300
	w13ms21	AY459297			w11cs01	DQ303222	cs01	DQ303220	040102	DQ303218
	w10cs01	DQ303230								
	cs02	DQ303229								
5–4	w08ms05	AY459299	0401	DQ303225	w09sn01	AF464059	0401	AF464051	0201	AY459300
	w13ms21	AY459297	0602	DQ303227	0401	DQ303223	0901	DQ303221	0801	DQ303219
	0401	DQ303228								

[a] Full-length *SLA-3* locus-specific primers failed to amplify products of desired size. *SLA-3* of this animal was amplified by alternative *SLA-3* primers modified from Ando et al. (2003). Partial coding sequences (495 bp) which included partial exon 1, exon 2 and partial exon 3 were obtained.

[b] *SLA-3* locus appeared to be homozygous in these animals. Second allele might have been missed due to preferential amplification of the full-length *SLA-3* primers or polymorphisms which interfere with primer binding.

Difficulties were encountered in the amplification of two of the *SLA-3* alleles with our usual full-length *SLA-3* locus-specific primers. Full-length *SLA-3**0101 and *SLA-3**0401 clones were found consistently in each of the animals that had the L and K haplotypes, respectively. Twenty-four *SLA-3* clones were screened for pig 5–4, only four of which yielded the *SLA-3**0602 allele. No full-length *SLA-3**0602 clones were found in two other pigs that had the N haplotype. The *SLA-3* alleles of pig MS failed to amplify with the full-length *SLA-3* locus-specific primers; however, partial coding sequences of the *SLA-3**0602 and *SLA-3**03cs01 alleles were obtained when the modified *SLA-3* locus-specific primers were used. The *SLA-3**03cs01 allele was only found in pig MS. No full-length sequence of this allele was found in two other pigs that had the M haplotype, despite 12 and 24 clones being screened for each pig.

SLA typing by PCR-SSP

Sequence-specific primers were designed that would distinguish each of the alleles present in all four haplotypes found in the Meishan pigs (Table 4). They were designed to have their 3′ nucleotides cover the polymorphic sequence motifs because sequence mismatches at the 3′ end of the primer would most likely interfere with the polymerase extension. Primers were also designed to have their melting temperatures only slightly lower than the annealing temperature of each PCR cycle to further increase their specificities. As described previously, positive control primers, which amplify a portion of the porcine α-actin gene, were multiplexed into each reaction to distinguish true negative reactions from false negative reactions (Martens et al. 2003).

Because only 19 alleles were observed at the five SLA loci in this Meishan herd, we used a 96-well setup to SLA genotype a maximum of four animals in each plate (Fig. 1). An abbreviated set of primers that distinguish each of the four *SLA-3* alleles and each of the three *SLA-DQB1* alleles for rapid haplotype assignment was also tested (picture not shown). Using this setup, a maximum of 12 animals can then be SLA typed in each plate.

All 20 samples from the breeding stock animals and the sample obtained from the University of Iowa were evaluated using this PCR-SSP method. The results were consistent with the sequence-based typing data as well as various combinations of the four Meishan haplotypes (Table 5). The four haplotypes, K, L, M, and N, were therefore assigned official haplotype numbers by the ISAG SLA Nomenclature Committee as Hp-4a.14, Hp-20.18, Hp-18.14, and Hp-19.15, respectively, with the number before the period representing the class I haplotype and the number after the period representing the class II haplotype (Table 6) (Smith et al. 2005a,b).

Discussion

In this study, we have successfully characterized the coding sequence of 19 SLA alleles defining four haplotypes at five

Table 4 Sequence-specific primers used to amplify Meishan SLA alleles in PCR-based genotyping and haplotyping assays

Lane number	Locus	Allele specificity	Primer ID	Position[a]	Forward and reverse primer sequence (5′→3′)	Product size
1	*SLA-DQB1*[b]	0801	#162	+166	CTTCAACGGAACGCAGCGGG	168 bp
			#163	+295	GCCCGCGTCTGCTCCAGGAT	
2		0201	#253	+186	GGGGCGTGGCCAGGTGG	177 bp
			#254	+319	GGTAGTTGTGTTTGCACACCSTGTCCAC	
3		040102	#147	+179	CAGCGGGTGTGGAGCGTGGA	197 bp
			#148	+327	TCCTCTATCTGGTAGTTGTGTTTGCACACA	
4	*SLA-DRB1*	0901	#212	+155	TGTCATTTCTTCAATGGGACCGAGCA	166 bp
			#213	+265	GCTCTAGGAGGTCCTTCTGGCTGTTATAGTT	
5		0401	#484	+126	CATTTCTTGTTTCTGGGGAAGGCT	205 bp
			#445	+289	CCGCCCGCTTCTGCTCCAT	
6		cs01	#330	+166	GGGACGGAGCGGGTGAGATTTC	163 bp
			#331	+289	CCGCCCGCTTCTGCTCCAT	
7	*SLA-1*	0401	#614	+123	GCCTGACCGCGGGGACTCT	181 bp
			#615	+261	GTAAGTCTGTGCGGTTTCCTTGACA	
8		w08ms05	#690	+130	TCGCTGGGACTCCCGCTTCT	123 bp
			#691	+212	GGCCCCTCCTGCTGTATCCACT	
9		w13ms21	#668	+360	GTCTCACACCCTCCAGAGCATGTTT	217 bp
			#618	+528	CAGTCCCTGCAGGTAGCTCCTCCTA	
10		w10cs01	#530	+404	CTCCTCCTCCGCGGGTACGA	180 bp
			#531	+544	CCACTCCACACACGTGCCCTC	
11		cs02	#469	+370	ACCTACCAGAACATGTACGGCTGCTACT	206 bp
			#1033	+526	CCCTGCAGGTAGCTCCTCTCTCC	
12	*SLA-2*	06me01	#1016	+207	CGCCCCGAATCCGAGGAAA	127 bp
			#1017	+292	CAGGGTGTTCAGGTTCACTCGGTA	
13		w09sn01	#668	+369	GTCTCACACCCTCCAGAGCATGTTT	214 bp
			#1021	+537	GCCTTGCAGGTAGCTCCTCCAG	
14		w11cs01	#801	+420	CCTCCGCGGGTACAGTCAGTTT	
			#632	+536	AGTCCCTGCAGGTAGCTCCTCCTAC	
15		0401	#239	+314	CCGAGGGAACCTGCGCACAGC	316 bp[c]
			#185	+362	CCCACGTCGCAGCCGTACATGA	
16	*SLA-3*[b]	03cs01	#826	+404	GCTCTTCCTCCACGGGTACCA	185 bp
			#476	+550	GGAGCCACTCCACACACGC	
17		0602	#848	+382	GCGACGTCGGGCCAGACT	154 bp
			#802	+502	GCATCGGCCGCCTCCCT	
18		0101	#862	+413	TCGCGGGTACAGTCAGTTTGG	217 bp
			#873	+590	TGCGTGCTGCAGCGTGTTAT	
19		0401	#675	+135	GGAAGCCCCGTTTCATCGAA	209 bp
			#361	+300	CTGGTTGTAGTAGCCGCGCAGGTTT	
All	α-Actin	Positive Control	#300	+21	CGCCATGTGTGACGAAGACGAGACC	516 bp[c]
			#301	+384	CACGTACATGGCGGGCACGTTGAAG	

[a] Referred to the 3′ end of the primers and relative to the start codon
[b] Primers of these loci were used in the abbreviated haplotyping assay
[c] Product spans intron

major loci in the current Meishan breeding stock maintained at the University of Illinois. The *SLA-DQA* and *SLA-DRA* loci were not characterized in this study due to the tight linkage to the *SLA-DQB1* and *SLA-DRB1* loci and the limited polymorphism of the *SLA-DRA* locus. In this study, we sequenced RT-PCR products rather than genomic DNA to obtain the entire coding sequence, to avoid the amplification of pseudogenes, and to ensure the genes are at least expressed at the mRNA level. To prevent or detect any amplification or sequencing artifacts, a high-fidelity polymerase was used, with multiple clones from either multiple pigs or multiple PCR amplifications being sequenced in both the forward and reverse directions.

We had difficulties in the amplification of the *SLA-3* alleles of the Hp-18.14 (M) and Hp-19.15 (N) haplotypes with our usual full-length *SLA-3* locus-specific primers. When we amplified the *SLA-3* alleles of pigs that have either of these haplotypes with the full-length *SLA-3* primers, preferential amplification of the allele from the other haplotypes was clearly observed. This primer pair

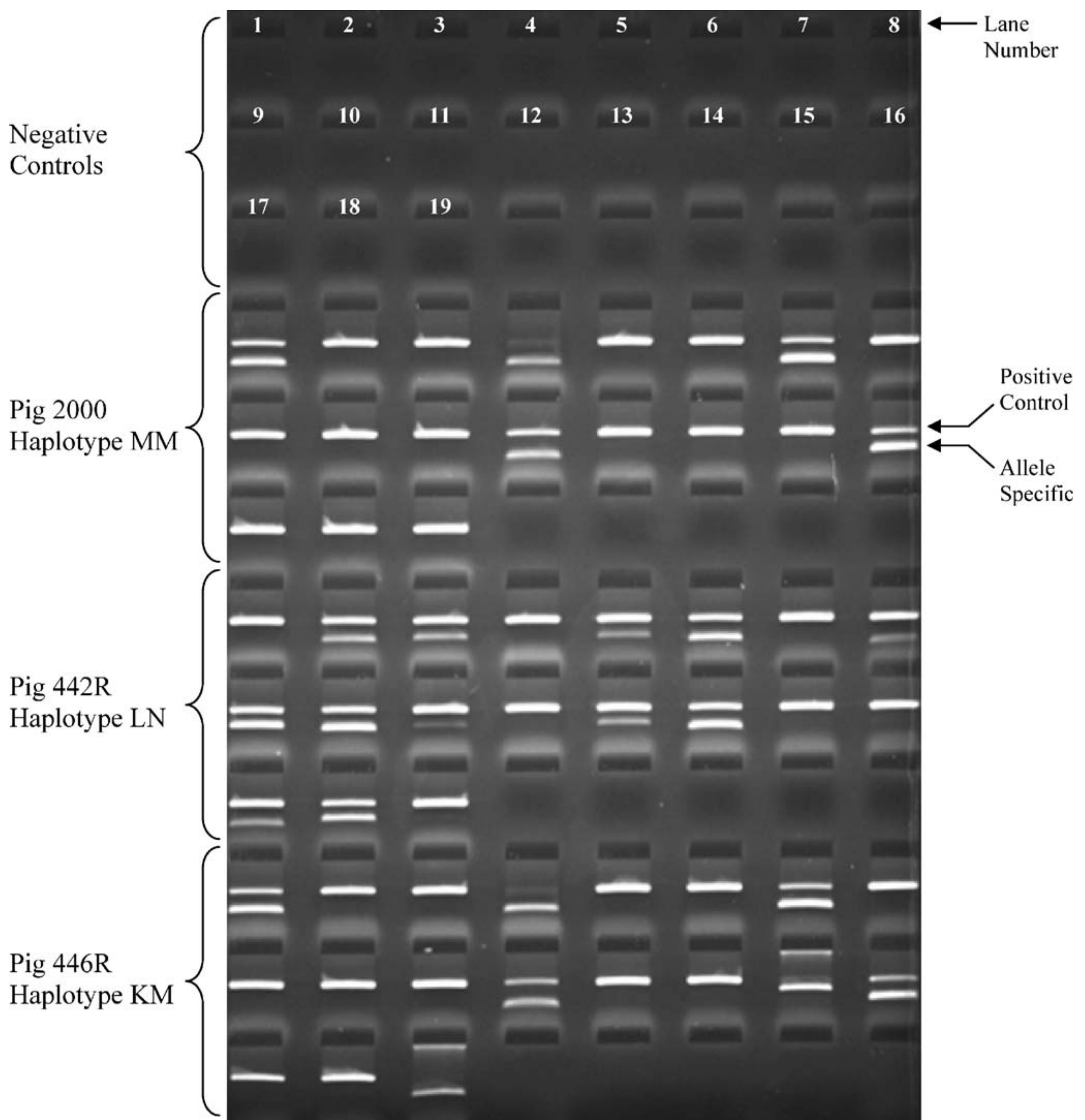

Fig. 1 Example of SLA genotyping of Meishan pigs at five loci (*SLA-DQB1*, *SLA-DRB1*, *SLA-1*, *SLA-2*, and *SLA-3*) by PCR-SSP using the full primer set (19 primer pairs, numbered 1 through 19, specific for each alleles found in the herd) (Table 4). Negative controls with all reagents except DNA were used to check for contamination. Positive control primers, which amplify a portion of the porcine α-actin gene (516 bp), were multiplexed into each reaction to check for adequate amplification. Allele-specific primers were designed to amplify products smaller than the positive control. The presence of a *smaller band* in a given lane indicates the pig is positive for that allele. Product sizes of each allele-specific primer are given in Table 4. Note that if both the positive control and the allele-specific band are absent, the reaction is considered to have failed and is not scored as either positive or negative. In some cases, the positive control band may be weak or absent in the presence of an allele specific product (e.g., *lane 19* for pig 446R). As long as either the positive control or the allele specific band is present, the reaction may be scored. *Lane 1 SLA-DQB1**0801. *Lane 2 SLA-DQB1**0201. *Lane 3 SLA-DQB1**040102. *Lane 4 SLA-DRB1**0901. *Lane 5 SLA-DRB1**0401. *Lane 6 SLA-DRB1**cs01. *Lane 7 SLA-1**0401. *Lane 8 SLA-1**w08ms05. *Lane 9 SLA-1**w13ms21. *Lane 10 SLA-1**w10cs01. *Lane 11 SLA-1**cs02. *Lane 12 SLA-2**06me01. *Lane 13 SLA-2**w09sn01. *Lane 14 SLA-2**w11cs01. *Lane 15 SLA-2**0401. *Lane 16 SLA-3**03cs01. *Lane 17 SLA-3**0602. *Lane 18 SLA-3**0101. *Lane 19 SLA-3**0401

Table 5 SLA haplotypes of the Meishan breeding stock maintained at the University of Illinois Imported Swine Research Center

Pig ID	Sex	SLA Haplotype	
		Local designation	ISAG nomenclature
MS[a,b]	M	M/N	18.14/19.15
1–13[b]	F	L/N	19.15/20.18
1–15	F	N/K	19.15/4a.14
2000	M	M/M	18.14/18.14
3000F	F	M/N	18.14/19.15
3000M	M	M/N	18.14/19.15
3001M	M	M/N	18.14/19.15
3001–0	M	M/N	18.14/19.15
368R	F	N/N	19.15/19.15
442R	F	L/N	19.15/20.18
446R[b]	F	M/K	18.14/4a.14
5–2	F	M/M	18.14/18.14
5–4[b]	F	N/K	19.15/4a.14
5–6	F	N/N	19.15/19.15
5–7	F	M/N	18.14/19.15
5001	M	N/N	19.15/19.15
5005	F	N/N	19.15/19.15
5010	F	M/N	18.14/19.15
5011[b]	F	L/M	18.14/20.18
5012	F	M/N	18.14/19.15
5013	F	M/N	18.14/19.15

[a] Sample provided by Dr. Zavazava at the University of Iowa (also derived from the University of Illinois herd)
[b] SLA characterized by cloning and sequencing of RT-PCR products

failed to amplify with the *SLA-3* alleles of the pig that had both of these haplotypes, in spite of multiple attempts with primer modifications and altered thermal cycling conditions. We had to use a modified primer pair based on the study by Ando et al. (2003) which only allowed us to obtain partial coding sequence of the alleles. This phenomenon of preferential amplification may be due to the polymorphism in the untranslated regions which in turn interfere with primer binding, or the relatively low expression of *SLA-3* mRNA transcript. Further studies to address these issues will lead to the improvement of specificity and efficiency of the locus-specific PCR amplification.

We have characterized the SLA types of 20 animals with potentially 40 haplotypes; however, we found only four different haplotypes. The Hp-18.14 and Hp-19.15 haplotypes are very common in the breeding stock. All of the animals have at least one of these two haplotypes. The Hp-4a.14 (K) and Hp-20.18 (L) haplotypes were less common with each being found in only three animals. It is possible that other herds of purebred Meishan pigs in the United States or China may have some additional haplotypes. This will require further screenings. The Hp-20.18 and Hp-19.15 haplotypes appear to have four class Ia loci. Phylogenetic analysis and PCR-SSP typing suggest that the *SLA-1* loci of these haplotypes have duplicated. In fact, *SLA-1* duplication has been reported previously in several haplotypes, such as the Hp-2.0 of the NIH miniature pigs and the Sinclair × Hanford crossbred melanoma pigs, the Hp-11.0 and Hp-12.0 of the Sinclair × Hanford crossbred melanoma pigs, and the Hp-8.0 of the Westran pigs (Smith et al. 2005a; Lee et al. 2005). These findings demonstrate that the number of MHC class Ia loci is haplotype-specific.

We have also developed a rapid PCR-SSP assay to SLA type any offspring in this herd. The full assay with complete primer set types alleles at all five loci. This approach will allow the selection of Meishan breeding stock to generate and maintain herds with defined SLA types. When the four SLA haplotypes of the parents are known, SLA haplotypes can be inferred by typing one SLA class I and one SLA class II locus because crossovers within the MHC regions are quite rare due to the tight linkage of the SLA genes. Vaiman et al. (1979) estimated the crossover between the class I and class II regions occurs in one out of 250 births while crossover between the *SLA-2* and *SLA-1/3* region occurs in one out of 2,000 births. To our knowledge, crossover within the class II region has never been documented. The abbreviated haplotyping assay, which types alleles at the *SLA-3* and *SLA-DQB1* loci, simplifies the typing of whole litters of pigs in a single run while maintaining its ability to detect any potential crossovers between the class I and class II region. It will most likely be used when selecting animals for experiments.

Table 6 ISAG assigned SLA haplotypes and alleles in Meishan pigs

Haplotype[a]	Local designation	Class I loci			Class II loci	
		SLA-1	*SLA-3*	*SLA-2*	*SLA-DRB1*	*SLA-DQB1*
Hp-4a.14	K	0401	0401	0401	0901	0801
Hp-18.14	M	0401	03cs01	06me01	0901	0801
Hp-19.15	N	w08ms05, w13ms21	0602	w09sn01	0401	0201
Hp-20.18	L	w10cs01, cs02	0101	w11cs01	cs01	040102

[a] The number before the period representing the class I haplotype and the number after the period representing the class II haplotype.

Numerous SLA typing methods have been described. Serologic typing using allo-antisera or monoclonal antibodies has been practiced extensively (Lunney 1994). However, there is limited availability of typing sera with well-defined specificities and no typing sera are available for many alleles, leading to many animals having untyped or “blank” antigens (Renard et al. 1988). Serologic typing also has inherent limitations on its ability to resolve alleles because serologic epitopes must be accessible on the surface of the SLA proteins. Many molecular-based techniques have also been documented, such as restriction fragment length polymorphism (RFLP) (Chardon et al. 1985), PCR-RFLP (Fang et al. 2005; Hosokawa-Kanai et al. 2002), and PCR-SSP (Ando et al. 2005; Martens et al. 2003). Ando et al. (2003) have described a similar SLA characterization approach by cloning and sequencing of RT-PCR products; however, their primer pairs were limited to certain groups of alleles and only partial coding sequence could be obtained. Haplotyping using microsatellite markers near the MHC region has been described as a surrogate test for SLA loci; however, the resolution of this method depends on the availability of markers in the region (Nunez et al. 2004; Tanaka et al. 2005).

Although SLA typing by cloning and nucleotide sequencing of RT-PCR products as described in this study is relatively labor intensive and technically demanding, it is the most direct and precise method for SLA typing. It has been used previously to characterize multiple breeds of pig, such as the NIH and Yucatan miniature pigs (Smith et al. 2005c), the Sinclair × Hanford crossbred melanoma pigs (unpublished data), the Westran pigs (Lee et al. 2005), and the Banna mini-pigs (Zeng and Zeng 2005). The PCR-SSP method described in this study has the limitation that it is based upon known nucleotide sequences and therefore may not detect new alleles. Nevertheless, it is fast, accurate, and easy to perform on a large scale. Using the same approach, we have also established SLA genotyping and haplotyping assays for the Sinclair × Hanford crossbred melanoma pigs (unpublished data), and the NIH and Yucatan miniature pigs (Martens et al. 2003).

In addition, we have already generated a collection of plasmid clones which contain various SLA genes with complete coding sequence from different breeds. These can potentially be used as reagents to synthesize SLA/peptide tetramers for the study of T-cell response to specific epitopes of certain endemic pathogens, such as the porcine reproductive and respiratory syndrome virus, as well as for the vaccine design.

Pigs have been used widely as an animal model for biomedical research (Schook et al. 2005). Given the intimate relationship between the MHC proteins and the immune system, resource herds of SLA-typed pigs are thus valuable tools for the study of disease resistance and vaccine responses in pigs. One of our main goals is to assist in the establishment of resource herds of SLA-defined pig for both agricultural research and biomedical science by characterizing their SLA diversity and polymorphism. This requires typing methodologies that are precise, relatively easy, and inexpensive, and able to SLA type outbred herds without knowing their genetic background. With the adoption of a formal SLA nomenclature system (Smith et al. 2005a,b), a publicly available SLA sequence database and the rapid acquisition of new SLA alleles and haplotypes we are gradually approaching this goal. Several resource populations of pig with well-characterized SLA antigens are now available worldwide for experiments in various disciplines, such as the Clawn miniature pigs, the NIH miniature pigs, the inbred Westran and Banna pigs, the Yucatan miniature pigs, and the crossbred Sinclair × Hanford melanoma pigs.

In conclusion, we have characterized the SLA diversity and polymorphism of a resource herd of Meishan pigs. We have also shown the combination of SLA typing methods by cloning and sequencing with PCR-SSP is a powerful tool to accurately characterize the SLA alleles of pedigreed pig populations.

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