

Table 1. Primer sequences, polymorphism descriptions and detection methods for *MC5R*.

SNP Position	Primer sequences	Annealing temp.	Type of polymorphism	Polymorphic fragments
Position 303 A ↔ G	F: 5'-TCA GCC TCT TGG AGA ACA TC-3' R: 5'-GCC ACC AAG GAG ATG CAG-3'	60 °C	PCR-RFLP (<i>Bsa</i> HI I)	allele 1: 238 bp allele 2: 179 and 59 bp
Position 841 C ↔ T	F: 5'-CCT CCA CCT CAT CCT GAT GAT TTC-3' R: 5'-TAT CAG AGG GTC GAT CAC G-3' R: 5'-TTA ATA ATT TTA TCA GAG GGT CGA TCA CA-3'	64 °C	Allele-specific PCR	allele 1: 128 bp allele 2: 118 bp

The porcine melanocortin-5 receptor (*MC5R*) gene: polymorphisms, linkage and physical mapping

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Source/description: The melanocortin-5 receptor mediates the effects of adrenocorticotrophic hormone (ACTH) or melanocortin stimulating hormones (MSH) on exocrine gland functions, including thermoregulation, immunomodulation, and sexual behaviour¹. Primers were designed in sequence regions conserved among the human, rat and bovine *MC5R* genes (Genbank accession nos U08353, L27081 and AJ002024, respectively). The consensus coding sequence (884 bp) of the porcine PCR products showed 82 % nucleotide identity and 81.2% amino acid identity to the corresponding human sequence. The porcine *MC5R* sequence has been submitted to GenBank (accession number AF133793). Two single nucleotide polymorphisms were found by sequence analyses of the PCR products amplified from the pooled DNAs of individual pigs from several different breeds.

Primer sequences:

PCR fragment A (680 bp)

Forward primer: 5'-TCAGCCTCTTGGAGAACATC-3'

Reverse primer: 5'-TTCCGCATCTCTTGGCTGC-3'

PCR fragment B (750 bp)

Forward primer: 5'-TCACCTGCA (A/C) TTCTGGATC-3'

Reverse primer: 5'-TGCTGGTCTCTGCCGAGCG-3'

PCR conditions: The PCR reactions were performed using 12.5 ng of porcine genomic DNA, 1.5 mM MgCl₂, 0.125 mM dNTP, 0.3 μM of each primer, and 0.35 U *Taq* DNA polymerase (Promega, Madison, WI) and PCR buffer (10 mM Tris-HCl, 50 mM KCl, and 0.1% Triton® X-100) in a 10-μl final volume. The PCR profile included 2 min at 94 °C; 35 cycles of 30 s at 94 °C, 1 min at 56 °C, 1 min and 30 s at

72 °C; and a final 15 min extension at 72 °C in a Robocycler (Stratagene, La Jolla, CA).

Polymorphisms: The *Bsa*HI PCR-RFLP and the allele-specific PCR were developed to test the polymorphisms at the positions 303 and 841, respectively (Table 1). PCR conditions for both polymorphisms were as described above, except for annealing temperatures (see Table 1). The allele specific PCR fragments and the PCR-RFLP products were separated by electrophoresis on 3% agarose gels (Fig. 1).

Mendelian inheritance/allele frequencies: Mendelian segregation of the allele-specific *MC5R* PCR products was observed in 3 three-generation PiGMap families². Allele frequencies of the two polymorphisms were obtained by genotyping 45 unrelated animals from several breeds in the Iowa State University herd (Table 2).

Chromosomal location/linkage: The porcine *MC5R* was assigned to chromosome 6q24-(1/2)q31 by PCR analysis of a pig-rodent somatic cell hybrid panel³. Two-point and multi-point linkage analyses were performed by genotyping the PiGMap families with the allele-specific PCR. The *MC5R* gene was most closely linked to *S0059* on SSC6 with (recombination frequency = 0.05 and LOD = 12.43). The best map order of the *MC5R* gene produced by the multi-point linkage analysis with other linked markers is as follows (with distance in Kosambi cM):

GPI-6.7- PGD-19.1-S0059-4.8-MC5R-6.9-ADCYAP1.

Comments: The assignment of the *MC5R* to the porcine chromosome 6 is in accordance with the previous results obtained by chromosome painting between human and pig⁴. The nucleotide substitution of porcine *MC5R* at the position 303 causes an amino acid change (Ala109Thr). The functional significance of this missense mutation is under investigation.

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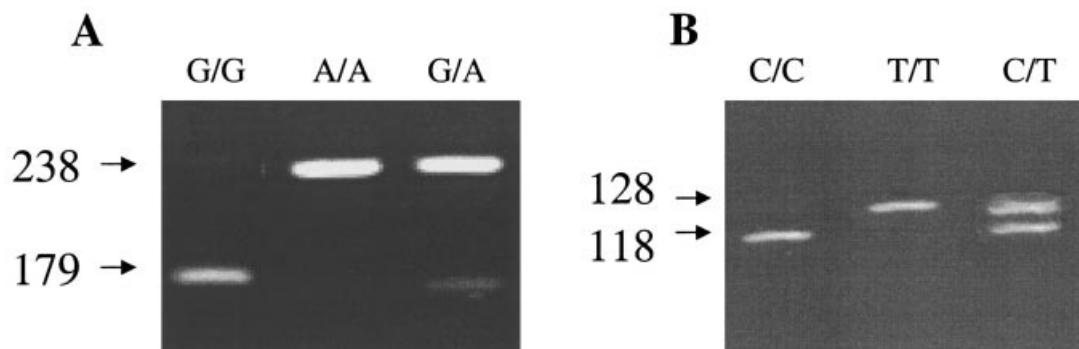


Fig. 1. Agarose gel (3%) showing genotypes of the porcine *MC5R* polymorphisms. Each genotype is indicated at the top of the lane. (A) The *Bsa*HI PCR-RFLP at position 303. The 59 bp fragment of allele 2 was not shown. (B) Allele-specific PCR polymorphism at position 841.

Table 2. Allele frequencies of the porcine *MC5R* polymorphisms in the Iowa State University herd.

Breeds	No. of animals	Position 303		Position 841	
		A	G	C	T
		Landrace	7	0.93	0.07
Hampshire	8	1	0	1	0
Duroc	8	0.75	0.25	1	0
Chester White	10	1	0	0.85	0.15
Yorkshire	8	1	0	0.875	0.125
Stress susceptible animals	6	0.58	0.42	1	0

References

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Nine polymorphisms within the head and hinge region of the feline cardiac β -myosin heavy chain gene

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Source/description: Nine polymorphisms were identified within six exons of the feline cardiac β -myosin heavy chain gene by evaluating DNA extracted from 20 cats from four different breeds, American Shorthair, British Shorthair, Maine Coon and domestic shorthair. DNA was extracted manually.¹ PCR primers were designed to amplify the 20 exonic regions between exons 3–23 using the exonic sequences of the feline β -myosin heavy chain gene (accession numbers AF001618–19, AF002811–2813, AF003767, AF005406–09).

Primer sequence:

Exon	Product size	Accession number
5 Forward TCTGTGTACCGTCAACCCTTAC	137 bp	AF175280
Reverse CAGCATGTACTGATAGGCGTTGTG		
8 Forward TGGAGGACCAGATCATCCAG	123 bp	AF001618
Reverse GCGGGAGGAGTTGTCCATCC		
13 Forward TGACAAGTCTGCCTACCTCATGG	119 bp	AF003767
Reverse ATTCTGCCCTTGGTGACGTAC		
14 Forward CCAAGGCAGTGTACGAGAAGATG	102 bp	AF005406
Reverse TGTCCAGGTCCCTATGAAGTATTG		
15 Forward CCAAGGCAGTGTACGAGAAGATG	113 bp	AF005407
Reverse ATGGGCTTCTCGATGAGTTCGATG		
16 Forward CATGGGCATCATGTCCATCC	126 bp	AF005408
Reverse GATATTCGTGGCTTCTGGAAGTTG		

PCR conditions and sequencing conditions: One hundred ng of feline genomic DNA were added to each PCR reaction (50 μ l total) of 25 μ l of water, 5 μ l of 10 \times PCR buffer, 250 μ M of each dNTP, 25 μ M of each primer and 0.2 μ l *Taq* polymerase (Amersham Life Sciences, Arlington Heights, IL). PCR reactions were performed at the following conditions: 94 °C for 5 min; 35 cycles of: 94 °C (60 s),

Table 1. Polymorphisms identified within the feline cardiac β -myosin heavy chain gene

Exon	Amino acid	Nucleotide	Codon	Codon
5	Proline	36	CCT	CCC
8	Threonine	6	ACC	ACT
8	Proline	36	CCT	CCC
13	Asparagine	87	AAC	AAT
14	Phenylalanine	51	TTC	TTT
14	Glutamine	87	GAG	GAA
15	Leucine	10	TTG	CTG
16	Proline	141	CCA	CCG

58 °C (60 s), 72 °C (90 s). The PCR product was electrophoresed on an ethidium bromide stained 2% agarose gel for 20 min at 100 W. The PCR product was excised from the gel for extraction, purified and sequenced using a Ampliqaq Dideoxy Sequencing kit on a ABI automated sequencer (Applied Biosystems, CA).

Polymorphisms: Nine polymorphisms were identified within six of the 20 exons evaluated. Polymorphisms were identified in exons 5, 8, 13, 14, 15 and 16. All polymorphisms were observed in the homozygous states only and none of the base pair changes affected the amino acid produced.

Chromosomal location: Unknown.

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References

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Linkage and physical mapping of the sheep perforin (*PRF1*) gene to OAR 25

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Source/description: Primers (SHPERF CCTCTgCACATTCTgTggA, SHPERR gATAggCgTCTgTggCTgTg) based on the second exon of the ovine perforin gene¹ were used to screen an ovine BAC library² resulting in the isolation of a single BAC clone (183C3R7). After digestion of the BAC clone with *Sau3aI*, size selected (400–600, 600–900, 900–1500 bp) libraries were constructed in an M13 vector, and screened with radioactively labelled (GT)₂₀ and (CAG)₈ oligonucleotides. Positive clones were sequenced and four dinucleotide and one trinucleotide repeats were identified. Oligonucleotide primers for four of the microsatellites were designed using the computer program PRIMER (The Whitehead Institute for Biomedical Research, MA, USA) to minimise self-annealing and to obtain a *T_m* of \approx 66 °C.

Primer sequences:

PERF3–2	JM173:	CAgggCTACACATgCATTgCT
	JM174:	TCAgCATATAgAgTTTCCTCATCTC
PERF4–1	JM175:	AgACTCggCACTgCCCAATACAT
	JM176:	TgggTggCTAAAgTgTTggAgCT
PERF5–3A	JM177:	gTACggCCCATAgCCATgTT
	JM178:	ggTtgAAACTgCTgTTCTTACACCT
PERFTRI	JM180:	gggTCgCAGAgTCAGACACCAT
	JM181:	AgCTggCAATATgAATgggCCT

Guanine nucleotides in the primer sequence are represented by lower case 'g' to ensure that they can be distinguished from cytosine 'C'. All primer sequences are listed 5'→3'. Limited amounts of these primers are available from J. Maddox.