A polymorphic porcine dinucleotide repeat S0559 (BHT0107) at chromosome 6q28-q31.

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MOLECULAR GENETIC MARKERS

A polymorphic porcine dinucleotide repeat *S0559* (BHT0107) at chromosome 6q28–q31

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Source/description: The sequences flanking a GT-repeat in porcine cosmid clone BHT0107 were sequenced with internal primers as described by Yuille *et al.*[1]

Primers:

5′-CCTGACATTTCTCACAAATATC-3′

5′-TCCAACTGATAATATGCAAGG-3′

PCR conditions: PCR amplifications were carried out in a volume of 20 μl containing 40 ng porcine DNA, 4 pmol each primer (one primer kinased), 0·65 mM each dNTP, 1× PCR buffer [45 mM Tris (pH 8·8), 11 mM ammonium sulphate, 1·5 mM $MgCl_2$, 6·7 mM β-mercaptoethanol, 4·5 μM EDTA] 0·5 U *Taq* polymerase. Thirty cycles were performed with each cycle consisting of 10 s at 94 °C, 30 s at 49·1 °C and 90 s at 70 °C.

Allele frequencies: The sizes of alleles were determined by comparison to M13mp18 DNA sequencing ladders (A and T). Allele frequencies were estimated from 25 unrelated pigs that constitute the PiGMaP grandparents[2] (Table 1).

Table 1 Allele frequencies

Allele (bp)	Frequency
170	0·18
172	0·02
176	0·56
178	0·2

Chromosomal localization: Fluorescence *in situ* hybridization (FISH) using 100–200 ng biotinylated DNA from cosmid BHT0107 to probe R-banded[3,4] male metaphase chromosome spreads revealed hybridization to the q31 band of chromosome 6 (FLpter 0·70–0·75, $n = 8$). No other chromosome region showed specific labelling of both homologues.

Mendelian inheritance: Co-dominant segregation of this polymorphism was observed in six three-generation families.

Comments: The sequence of *S0559* has been submitted to GenBank (accession number: AF034215).

References

1 Yuille M.A.R. *et al.* (1991) *Nucl Acids Res* **19**, 1950.
2 Archibald A.L. *et al.* (1995) *Mamm Genome* **6**, 157–75.
3 Lemieux N. *et al.* (1992) *Cytogenet Cell Genet* **59**, 311–2.
4 Thomsen P.D. *et al.* (1993) *Mamm Genome* **4**, 604–7.

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A polymorphic porcine dinucleotide repeat *S0560* (BHT0491) at chromosome 6q21–q22

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Accepted 17 December 1997

Source/description: The sequences flanking a GT-repeat in porcine cosmid clone BHT0491 were sequenced with internal primers as described by Yuille *et al.*[1]

Primers:

5′-TCAGACCCAGCAACATGCC-3′

5′-TCCCGAGTAGCCTTGTGTC-3′

PCR conditions: PCR amplifications were carried out in a volume of 26 μl containing 50 ng porcine DNA, 5 pmol each primer, 0·125 pmol kinased primer, 0·5 mM each dNTP, 1× PCR buffer, 1·25 μl DMSO, 1 U *Taq* polymerase. Thirty cycles were performed with each cycle consisting of 30 s at 94 °C, 30 s at 60 °C and 30 s at 72 °C.

Allele frequencies: The sizes of alleles were determined by comparison to M13mp18 DNA sequencing ladders (A and T). Allele frequencies were estimated from 22 unrelated pigs that constitute the PiGMaP grandparents[2].

Chromosomal localization: Fluorescence *in situ* hybridization (FISH) using 100–200 ng biotinylated DNA from cosmid BHT0491 to probe R-banded[3,4] male metaphase chromosome spreads revealed hybridization to the q21–q22 region of chromosome 6 (FLpter 0·35–0·44, $n = 8$) and to the q21 region of pig chromosome 2. As linkage analysis in the PiGMaP[2] pedigrees excluded linkage to markers on pig chromosome 2, we assign *S0560* to 6q21–q22.

Table 1. Allele frequencies

Allele (bp)	Frequency
201	0·05
203	0·30
205	0·52
207	0·05
209	0·09

Mendelian inheritance: Co-dominant segregation of this polymorphism was observed in six three-generation families.

Comments: The sequence of *S0560* has been submitted to GenBank (accession number: AF034216).

References

1 Yuille M.A.R. *et al.* (1991) *Nucl Acids Res* **19**, 1950.
2 Archibald A.L. *et al.* (1995) *Mamm Genome* **6**, 157–75.
3 Lemieux N. *et al.* (1992) *Cytogenet Cell Genet* **59**, 311–2.
4 Thomsen P.D. *et al.* (1993) *Mamm Genome* **4**, 604–7.

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Molecular markers for the bovine fibrillin 1 gene (*FBN1*) map to 10q26

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Source/description: A bovine cDNA of about 1 kb encoding fibrillin l (*FBN1*), cloned into pBluescript, was used directly for fluorescence *in situ* hybridization (FISH) mapping and for the screening of a six-

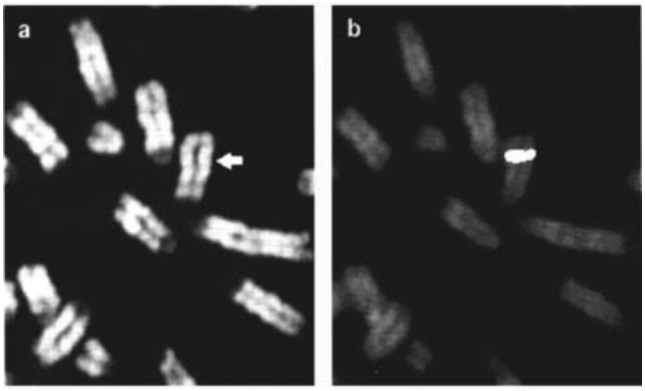

Fig. 1. Partial bovine metaphase spread before (a; QFQ-banded) and after (b; DAPI-counterstained) FISH with a YAC positive for bovine *FBN1*. The arrow indicates chromosome 10.

genome equivalent bovine YAC library arrayed at high density (A. Schoeberlein, in preparation). Two positive YACs were isolated.

Chromosomal mapping: The plasmid containing the cattle *FBN1* cDNA and one of the positive YACs were mapped by FISH to cattle chromosomes as described by Solinas Toldo *et al.*[1] However, to obtain a specific signal with the plasmid probe it was necessary to apply 1 μg of labelled DNA on a 18 × 18 mm^2 slide area. One microgram of total yeast DNA was also used for YAC–FISH. In both cases specific signals were detected on chromosome 10q26 (FL_{cen} 0·6 ± 0·025) (Fig. 1).

Comments: The bovine Marfan syndrome is a disorder similar in its clinical characteristics to the human Marfan syndrome, which is caused by mutations in the type 1 fibrillin. Fibrillin 1 is therefore a candidate gene for the bovine syndrome[2]. The *FBN1* gene was localized to human chromosome (HSA) 15q21·1[3] and to mouse chromosome (MMU)2[4]. The cattle *FBN1* has been assigned by the analysis of a bovine × rodent cell panel to syntenic group U5 (BTA10). The localization of *FBN1* to chromosome 10 could also be expected since an extended segment of HSA15 was shown to be conserved in cattle chromosome BTA10. However, the assignment of *FBN1* to band q26 is one band distal of the HSA15-conserved segment as demonstrated with a HSA15 painting probe[5]. This discrepancy may be due to the relatively low resolution achieved by the heterologous chromosome painting.

References

1 Solinas Toldo S. *et al.* (1995) *Cytogenet Cell Genet* **69,** 1–6.
2 Tilstra D.J. *et al.* (1994) *Genomics* **23,** 480–5.
3 Magenis R.E. *et al.* (1991) *Genomics* **11,** 346–51.
4 Li X. *et al.* (1993) *Genomics* **18,** 667–72.
5 Solinas Toldo S. *et al.* (1995) *Genomics* **27,** 489–96.

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Characterization of 10 polymorphic alpaca dinucleotide microsatellites

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Source/description: The microsatellites VOLP-03, -05, -08, -10, -12, -17, -32, -33, -59 and -67 were isolated from an alpaca (Camelidae: *Lama pacos*) library, prepared on the pLITMUS 28 (Promega, Madison, WI, USA) vector, ligating *Bgl*II/*Bcl*I/*Bam*HI-digested genomic DNA fragments of 500–800 bp into the *Bam*HI-digested plasmid, according to Lund *et al.*[1] The library was screened using a 24-mer probe [poly(dA-dC)(dG-dT), Pharmacia Biotech, Uppsala, Sweden], digoxigenin labeled using the DIG oligonucleotide 3′-end labeling kit and revealed using the Genius System (Boehringer Mannheim, Mannheim, Germany). The clones giving a clear positive signal after a second hybridization were sequenced on an ABI 377A sequencer, using the M13/pUC sequencing primers (NE Biolabs, Beverly, MA, USA) and the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit. PCR primers were designed using the Oligo 5·0 program.

Table 1. Primer sequences

Locus	Primer sequence (5′–3′)
VOLP-03	AGACGGTTGGGAAGGTGGTA
	CGACAGCAAGGCACAGGA
VOLP-05	ACTTAATCACCTGGATGTAT
	ATATGGTTCACTGTGTTTACT
VOLP-08	CCATTCACCCCATCTCTC
	TCGCCAGTGACCTTATTTAGA
VOLP-10	CTTTCTCCTTTCCTCCCTACT
	CGTCCACTTCCTTCATTTC
VOLP-12	TTGTTCTCAACAGGGACTGC
	TCTGGCCCACCCACTAA
VOLP-17	GACTTCCAGCCACAAC
	CCATCAGTTTTAAGCAACATAC
VOLP-32	GTGATCGGAATGGCTTGAAA
	CAGCGAGCACCTGAAAGAA
VOLP-33	TAATCCTTAAGAACCGACCCT
	GGACCCGCTTCATCTATTG
VOLP-59	CCTTCCTCAGAATCCGCCACC
	CCCGCGCACCAAGCAG
VOLP-67	TTAGAGGGTCTATCCAGTTTC
	TGGACCTAAAAGAGTGGAG

PCR conditions: Reactions were made in 25 μl volumes on buffer #6 (Opti-primer PCR optimization kit, Stratagene, La Jolla, CA, USA) containing 75 mM KCl, 10 mM Tris–HCl (pH 8·8), 1·5 mM $MgCl_2$, plus 0·2 mM each dNTP's, 0·2 μM each primer (one of them labeled with a fluorochrome), 0·5 U *Taq* polymerase (Boehringer Mannheim) and 50 ng template DNA. After an initial denaturation step at 95 °C for 4 min, reactions were run 30 cycles in a PTC-100 thermocycler (MJ Research, Watertown, MA, USA), each cycle including a denaturation step at 94 °C for 1 min, an annealing step at 55 °C for 1 min and an extension step at 72 °C for 1 min. Primers are described in Table 1.

Polymorphism: A group of 36 alpacas were analyzed using the ABI GeneScan software. Information regarding the locus names, repeat characteristics and allele number and size range, as well as the expected heterozygosity and PIC values, are shown in Table 2.

Table 2. Summary data for loci and primers

Locus	Repeat	Alleles (*n*)	Size range (bp)	Hetero-zygosity	PIC
VOLP-03	$(TG)_{13}$	8	129–169	0.799	0.74
VOLP-05	$(TG)_{13}$	6	134–150	0.79	0.753
VOLP-08	$(TG)_{10}TCCG(TG)_2TCCG(TG)_5$	3	148–152	0.226	0.214
VOLP-10	$(TG)_2TA(TG)_7TA(TG)_7$	3	231–235	0.596	0.521
VOLP-12	$(TG)_3CG(TG)_4CG(TG)_7$ $CT(TG)_2CA(TG)_2$	2	136–148	0.08	0.077
VOLP-17	$(GA)_5CAGATACAGA(TA)_2$ $GATTT(TG)_{14}(TTG)_4TG$	12	184–228	0.784	0.733
VOLP-32	$(TG)_{20}$	12	192–247	0.797	0.802
VOLP-33	$(A)_4G(A)_5(TG)_{12}T(A)_5$	4	178–184	0.676	0.591
VOLP-59	$(TG)_7[(CG)_2(TG)_5]_2(CG)_2$ $(TG)_4CT(TG)_4AGCT(TG)_3$	5	112–136	0.683	0.614
VOLP-67	$(TG)_5(G)_4(TG)_9CG(TG)_7$	5	158–170	0.671	0.584

Chromosomal location: Not known.

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Reference
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Two highly polymorphic microsatellites within the porcine secretory carrier membrane protein 1 (*SCAMP1*) gene

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Source/description: Secretory carrier membrane proteins (SCAMPs) are components of secretory vesicle membranes that function as recycling carriers in the secretory pathway. SCAMPs are widely distributed and present in almost all mammalian tissues and cell types and play a fundamental role in the general cell surface recycling. We have isolated a cDNA coding for the SCAMP1 mRNA and determined the genomic structure of the porcine *SCAMP1* gene by isolation and sequencing of seven recombinant phage clones. Within intron 4 (phage λ7a) and intron 7 (phage λ35) of the *SCAMP1* gene an $(AT)_{10}$ and $(GT)_{23}$ dinucleotide repeat was identified, respectively. The DNA sequences of the *SCAMP1* gene were submitted to the EMBL data library under accession numbers AJ223737, AJ223738, AJ223740, and Y15710.

Primer sequences:

Forward primer (intron 4): 4F
5′-CAG AAC TGA GGC TAA AGT AC-3′

Reverse primer (intron 4): 4R
5′-CAG AGT TGT AGG ACT GTA GAG-3′

Forward primer (intron 7): 7F
5′-CAG CTC CAC TTC GAC TCC T-3′

Reverse primer (intron 7): 7R
5′-TTG AAG AGT GGA GTC ACT GC-3′

PCR conditions: PCR primers were designed from the DNA sequences of the flanking regions. PCR reactions were optimized using the 'Ready to Go' system (Pharmacia, Freiburg, Germany). A master mix was prepared containing 400 mM of each primer (the reverse primer was labelled with IRD800 fluorescence dye; MWG Biotech, Ebersberg, Germany), frozen and stored at –20 °C. DNA (100 ng) in 1 μl TE was mixed with 12 μl master mix and one PCR bead was added (10 mM Tris–HCl pH 9·0, 50 mM KCl, 1·5 mM $MgCl_2$, 200 mM of each dNTPs, and 1·5 U *Taq* polymerase). Cycling included an initial denaturation step at 94 °C for 10 min, followed by 30 cycles of 1 min at 94 °C, 30 s at 55 °C, 1 min 72 °C for and a final step of 10 min at 72 °C using a Hybaid Omnigene (Ashford, UK) cycler. PCR products were analyzed by electrophoresis on 8% denaturing polyacrylamide gels using a LI-COR Gene ReadIR 4200 automated sequencer. Analysis of the bands was performed using the RFLPscan Plus 3·0 (Scanalytics, Virginia, VA, USA) software.

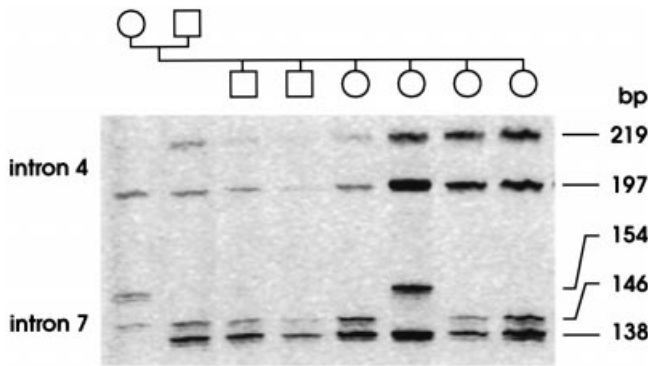

Fig. 1. Multiplex PCR analysis of the dinucleotide repeats in intron 4 and intron 7 of the *SCAMP1* gene showing Mendelian inheritance. Allele sizes are indicated.

Table 1. Polymorphic information content, heterozygosity, allele sizes and frequencies of the two *SCAMP1* gene microsatellites

	Intron 4	Intron 7
Repeat sequence	$(AT)_{10}$	$(GT)_{23}$
PIC	0.84	0.76
Heterozygosity	0.85	0.78
Allele size* (frequency)	219 (0.08)	158 (0.03)
	207 (0.02)	156 (0.01)
	205 (0.06)	154 (0.03)
	203 (0.13)	150 (0.06)
	201 (0.16)	148 (0.06)
	199 (0.24)	146 (0.36)
	197 (0.07)	138 (0.09)
	195 (0.13)	136 (0.06)
	193 (0.11)	134 (0.07)
		132 (0.23)

*Allele size in bp.

Chromosomal localization: The porcine *SCAMP1* gene has been assigned to chromosome 2q21–q22 by FISH analysis and PCR screening of a porcine-rodent somatic hybrid cell panel[1].

Mendelian inheritance: Mendelian inheritance was followed in 48 individuals from five families of Angler Saddlebacks (Fig. 1).

Polymorphism: Polymorphisms were studied using DNA samples of 50 Angler Saddleback, five German Large White, five German Landrace, five Piétrain, and six Bunte Bentheimer pigs. Among these 71 pigs, nine different alleles of the microsatellite in intron 4 were detected. The lengths of the corresponding PCR products were 193, 195, 197, 199, 201, 203, 205, 207, and 219 bp. Using the same individuals, the microsatellite in intron 7 showed 10 different alleles with lengths of the PCR products of 132, 134, 136, 138, 146, 148, 150, 154, 156, and 158 bp. Heterozygosity, PIC values and allele frequencies are shown in Table 1.

Acknowledgements: The authors are grateful to A. Deppe for expert technical assistance.

Reference
1 Yerle M. *et al.* (1997) *Cytogenet Cell Genet* **73,** 194–202.

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Characterization of a polymorphism in exon 1 of the porcine hormone-sensitive lipase (*LIPE*) gene

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Source/description: Degenerate primers were designed using human, mouse and rat sequences of the hormone-sensitive lipase (*LIPE*) gene (GenBank accession numbers L11706, U08188, X51415, and J03087). The primers were used to amplify a 498-bp fragment of putative exon 1 (coding region) of porcine *LIPE*.

Primer sequences:

Forward: 5′ CGCACRATGACACAGTCGCTGGT 3′

Reverse: 5′ CAGGCAGCGRCCRTAGAAGCA 3′

(R stands for A and G)

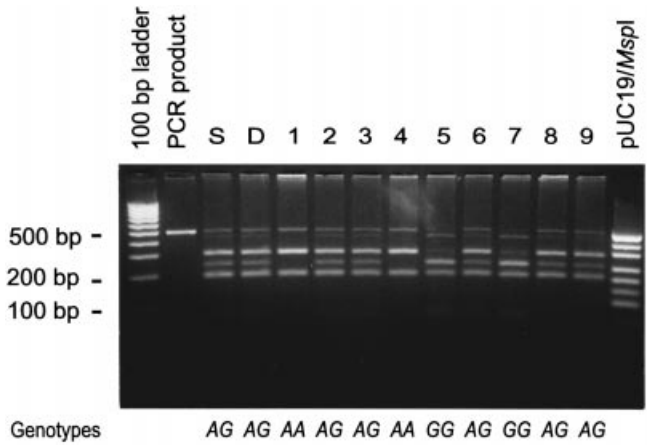

Fig. 1. Mendelian inheritance of the *Hsp*92I polymorphism at the porcine *LIPE* gene (allele *A* – two predominant bands, 308 and 190 bp; allele *G* – two predominant bands, 241 and 190 bp; the 67 bp band is barely visible). The weak zones, which do not obscure the genotypes, are apparently due to the incomplete digestion that could not be avoided. The 100 bp ladder has bands 1000–100 bp, and pUC19/*Msp*I marker possesses bands 501/489, 404, 331, 242, 190, 147, 111/110, 67, 34, and 26 bp. S, sire; D, dam; 1–9, offspring.

PCR conditions/cloning/sequencing: PCR was performed in 50 μl reactions using 100 ng porcine genomic DNA, standard PCR buffer, 2 mM $MgCl_2$, 200 μM each dNTP, 0·4 μM each primer and 1·3 U *Taq* polymerase. After an initial 95 °C denaturation step (2 min) the PCR was carried out at 95 °C (45 s), 60 °C (1 min) and 72 °C (1 min; the final extension 5 min) for 30 cycles. Amplification products were electrophoresed on a 4% NuSieve agarose gel. A single fragment of the expected size was observed. The PCR products generated from two crossbred pigs (Meishan × Piétrain and Wild Boar × Piétrain) were ligated into pUC18, and four recombinant clones were sequenced using an ALFexpress Sequencing System (Pharmacia Biotech, Uppsala, Sweden). The sequence (EMBL accession number AJ224692) was almost identical to the recently available data on porcine *LIPE* (EMBL accession number AJ000482).

Polymorphism: Comparison of the sequences of the four clones of porcine *LIPE* revealed a $^{433}A \rightarrow G$ polymorphism (numbering from the 5′ end of the PCR fragment), which was found to be present within a recognition sequence for enzyme *Hsp*92I. Digestion of the PCR product with the enzyme revealed two alleles: *A* (fragments 190 and 308 bp) and *G* (fragments 190, 241 and 67 bp). The three genotypes observed are shown in Fig. 1. The substitution of $^{433}A \rightarrow G$ also results in an amino acid substitution (**A**TC – Ile; **G**TC – Val) in the corresponding protein.

Mendelian inheritance/allele frequencies: Codominant inheritance of the *LIPE* alleles was demonstrated in the Hohenheim PiGMaP Meishan × Piétrain three-generation pedigree[1]. Both alleles were present in Meishan; in Piétrain allele *G* was fixed. Unrelated animals of Landrace ($n = 8$) and Large White ($n = 7$) breeds were monomorphic for allele *G*, while Duroc ($n = 11$) was polymorphic (frequencies: *A* – 0·23; *G* – 0·77).

Chromosomal location: The *LIPE* gene has been assigned by FISH to porcine chromosome 6q12[2].

Acknowledgements: We thank Professor H. Geldermann for providing DNA samples from the Hohenheim PiGMaP pedigrees. This study was supported by Grant Agency of the Czech Republic (Grant no. 523/97/1305) and EC (Contract no. ERBIC15CT960902).

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2 Chowdhary B.P. *et al.* (1994) *Cytogenet Cell Genet* **67,** 211–4.

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Three new microsatellite markers on bovine chromosome 17

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Source/description: A YAC clone, mapping to bovine chromosome 17 was digested with *Mbo*I and subcloned into pUC18. The resulting plasmid sublibrary was screened with oligolabeled d(CA)-d(GT) repeat probe and the positive transformants isolated. Three strongly hybridizing microsatellite containing clones were sequenced and PCR primers chosen from the repeat flanking regions. One primer of each pair was labeled with a fluorescent tag.

PCR conditions: Amplifications were carried out in microtitre plates in 10 μl final volumes. Each reaction contained 10–20 ng genomic DNA, 200 μM each dNTP, 0·25 μM corresponding primers, 2 mM $MgCl_2$, 0·125 U *Tth* polymerase (Epicentre Technologies, Madison, WI, USA) and 1× reaction buffer. Thermocycling consisted of initial denaturation at 95 °C for 5 min, followed by 40 cycles of 10 s at 95 °C denaturation, 40 s at the specific annealing temperature and 20 s extension at 72 °C. Each reaction was quantitated by running 4 μl product on 3% agarose gel and the remainder of the samples diluted, aliquoted and pooled. The pooled DNA fragments were separated on an ABI 373 automatic sequencer. Allele sizes and genotypes were estimated by Genescan 2·1 analysis software.

Synteny mapping: The chromosomal locations of the new markers were found by PCR typing of a mouse/cattle hybrid cell panel[1]. The three microsatellites scored statistically significant syntenies between themselves and with other markers on chromosome 17.

Mendelian inheritance and linkage: Codominant segregation was observed in the International Bovine Reference Families[2]. The three microsatellites are polymorphic, showing different levels of polymorphism. Allele sizes and the number of alleles for each marker are listed in Table 1, as they were found in the typed families. Allele frequencies were calculated from the 38 parental genotypes only, assuming no close relationships between the animals. As expected, the three markers are tightly linked and map within the pericentromeric region of chromosome 17.

Table 1. Primer information, allele sizes and levels of polymorphism

Marker accession no.	Primer sequences	T°a (°C)	Allele sizes (bp) and frequencies								
AF6	GAATGGCCAAGAGAACACATACAGAACATC	61	230	240	242	244					
Y15071	CCCCTGGAGGAGGGCATGGC		(0·013)	(0·316)	(0·276)	(0·395)					
AF7	GGACAGAGGAGCCTGGCAGGG	62	177	187	189	191					
Y15072	AATGAATGGCCAAGAGAACACATACAGAAC		(0·013)	(0·316)	(0·276)	(0·395)					
AF8	GATCCACAGGGTCGCCAAGAGTTG	62	164	166	168	170	172	174	176	178	180
Y15073	TTGTCTTACAGGAGGAGATGAGCACATGTC		(0·066)	(0·026)	(0·290)	(0·211)	(0·092)	(0·065)	(0·105)	(0·013)	(0·132)

Allele frequencies are shown in parentheses under the corresponding alleles.

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2 Barendse W. *et al.* (1997) *Mamm Genome* **8,** 21–8.

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A polymorphic horse microsatellite locus: VHL150

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Source/description: A plasmid clone was isolated from a library of size-selected horse fragments following screening with a $(CA)_{25}$ oligonucleotide. The sequence of this clone which contains a $(AC)_{14}$ microsatellite was determined. The sequence of this locus has been submitted to EMBL data library (accession number X86450).

Primer sequences:

5′-CTCCTATGGGTGTCAGAAAG-3′ (forward)

5′-TGTAATCTGTGGGGGAGACT-3′ (reverse)

PCR conditions: As a template for PCR amplification 75–125 ng DNA was used in a total reaction volume of 15 μl, containing 10 mM Tris–HCl (pH 8·3), 50 mM KCl, 1·5 mM $MgCl_2$, 5 pmol of each primer (Perkin Elmer, Foster City, CA, USA) and 1·2 U Amplitaq (Perkin Elmer). PCR amplification was carried out on a Perkin Elmer GeneAmp PCR system. Thirty cycles of 10 s 94 °C, 10 s 55 °C, and 10 s 72 °C, were performed.

Allelic frequencies: The sizes of allelic PCR products were determined with the ABI 373 DNA sequencer and Genescan software (Perkin Elmer-ABI, Foster City, CA, USA). The frequencies of the microsatellite alleles are based on samples submitted for parentage control. Frequencies are presented for Dutch Warmblood, Fjord Horse, Friesian Horse, Shetland Pony, and Icelandic Horse (Table 1).

Table 1. Allelic frequencies, PIC, heterozygosity and PE values for VHL150

	Breeds				
	DW	FjH	FrH	SP	IH
Alleles (bp)	*n* = 1350	*n* = 650	*n* = 25	*n* = 10	*n* = 12
96	0·158	0·424	0·059	0	0·095
98	0	0·064	0	0	0
100 (M*)	0·654	0·415	0·137	0·214	0·476
102	0·163	0·093	0·471	0·714	0·429
104	0·008	0·005	0	0	0
106	0·016	0	0·333	0·071	0
PIC	0·475	0·565	0·581	0·387	0·490
Heterozygosity	0·520	0·636	0·439	0·439	0·586
PE	0·291	0·363	0·218	0·218	0·286

DW, Dutch Warmblood; FjH, Fjord Horse; FrH, Friesian Horse; SP, Shetland Pony; IH, Icelandic Horse.
*In the international nomenclature for parentage control with horse microsatellites, base pair values of alleles are renamed to and presented in an alphabetical order (e.g. 98 bp = 'L', 100 bp = 'M', 102 bp = 'N', etc.).

Mendelian inheritance: Transmission of the microsatellite alleles was consistent with codominant, autosomal inheritance.

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A highly polymorphic microsatellite within intron 5 of the porcine 54/56 kDa vacuolar H(+)-ATPase subunit gene (V-ATPase)

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Accepted 1 July 1998

Source/description: The vacuolar proton-translocation ATPases are multienzyme complexes located in the membranes of eukaryotic cells regulating the cytoplasmic pH. We have isolated and characterized a cDNA coding for the porcine 54 and 56 kDa subunit of the V-ATPase. The genomic structure was determined by sequencing of recombinant phage clones and assigned it to chromosome 4q15–q16 by PCR screening of a porcine-rodent hybrid cell panel. The V-ATPase gene consists of 14 exons with sizes ranging from 90 to 336 bp with a non-coding first exon and an alternatively spliced seventh exon. Within the fifth intron of the V-ATPase gene a $((CA)_{13}*(CT)_{17})$ dinucleotide repeat was identified. The DNA sequence of the V-ATPase gene was submitted to the EMBL data library under accession number AJ223748.

Primer sequences:

Forward primer (EXO 6f): 5′-CCA CAC ACT TGC TAA CAG AAA-3′

Reverse primer (EXO 6r): 5′-CAC TGC AAT AAA TCT GGA AGA G-3′

PCR conditions and analysis: PCR reactions were optimized using the 'Ready to Go' system (Pharmacia, Freiburg, Germany). A master mix was prepared containing 400 μM of each primer (the forward primer was labelled with IRD 800 fluorescence dye; MWG Biotech, Ebersberg, Germany), frozen and stored at –20 °C. DNA (100 ng) in 1 μl TE (10:1) was mixed with 12 μl master mix and one PCR bead was added (10 mM Tris–HCl pH 9·0, 50 mM KCl, 1·5 mM $MgCl_2$, 200 μM of each dNTP and 1·5 U *Taq* polymerase). Cycling included an initial denaturation step at 94 °C for 10 min, 30 cycles of 1 min at 94 °C, 30 s at 55 °C, 72 °C for 1 min and a final step of 10 min at 72 °C using a Hybaid Omnigene cycler. PCR products were analyzed by electrophoresis on 8% denaturing polyacrylamide gels using a LI-COR Gene ReadIR 4200 automated sequencer. Analysis of the bands was performed using the RFLPscan Plus 3·0 (Scanalytics, Virginia, VA) software.

Polymorphism: Polymorphism was studied using DNA samples of

Table 1. Polymorphic information content, heterozgosity, allele sizes and frequencies of the V-ATPase gene microsatellite

Repeat sequence	$(CA)_{13}*(CT)_{17}$
PIC	0·91
Heterozygosity	0·92
Allele size (bp) and frequency	227 (0·028)
	225 (0·011)
	223 (0·011)
	221 (0·017)
	219 (0·011)
	217 (0·011)
	215 (0·086)
	213 (0·022)
	211 (0·011)
	209 (0·006)
	207 (0·051)
	205 (0·028)
	201 (0·022)
	199 (0·086)
	197 (0·115)
	195 (0·033)
	193 (0·057)
	191 (0·068)
	189 (0·057)
	185 (0·167)
	183 (0·051)
	181 (0·051)

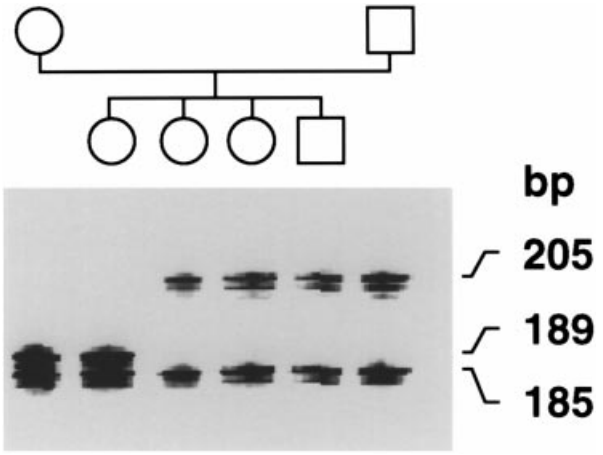

Fig. 1. PCR analysis of the dinucleotide repeat in intron 5 of the V-ATPAse subunit gene demonstrating Mendelian inheritance. Allele sizes are indicated.

77 Angler Saddleback, five German Large White, five German Landrace, five Pietrain, and six Bunte Bentheimer pigs. Among these 98 pigs 22 different alleles of the microsatellite were detected. The lengths of the corresponding PCR products were 227, 225, 223, 221, 219, 217, 215, 213, 211, 209, 207, 205, 201, 199, 197, 195, 193, 191, 189, 185, 183, and 181 bp. Heterozygosity, PIC values, and allele frequencies are shown in Table 1.

Mendelian inheritance: Codominant segregation of the polymorphisms was observed in 48 individuals from five families of Angler Saddlebacks (Fig. 1).

Chromosomal localization: The porcine V-ATPase gene was assigned to chromosome 4q15–q16 by PCR screening of a porcine rodent somatic cell hybrid panel[1].

Acknowledgements: The authors are grateful to A. Deppe for expert technical assistance.

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Omy0002DIAS: a highly polymorphic dinucleotide microsatellite in rainbow trout (*Oncorhynchus mykiss*)

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Accepted 28 March 1998

Source/description: A genomic plasmid library was constructed in the plasmid vector pCR-Script (Stratagene, La Jolla, CA) from size-selected (300–800 bp) rainbow trout genomic DNA digested with *Pvu*II and *Hae*III. The library was screened with a $(TG)_9$ probe. Positive clones were sequenced using an automatic DNA sequencer (ALF express DNA Sequencer, Pharmacia, Uppsala, Sweden). The clone Omy0002DIAS was shown to contain a $(TG)_{28}$ repeat (GenBank accession number AF039065). PCR primers were designed on the basis of the flanking sequences. The sizes of the PCR fragments were analyzed on the ALF express DNA Sequencer.

Primer sequences:

5′-AGGCTAACATCGAACTATCAAT-3′

5′-CTGTGGTCTGGTTCTGTTCA-3′

PCR conditions: Approximately 40 ng genomic DNA was amplified in a total volume of 25 μl. The reaction contained 5 pmol of each primer, 0·1 mM dNTP, 0·4 U *Taq* polymerase (Pharmacia), 2·5 mM $MgCl_2$, 50 mM KCl, and 10 mM Tris–HCl. Amplification was carried out using a Touchdown Temperature Cycler (Hybaid, Teddington, Middlesex, UK). Reaction conditions were an initial denaturation step at 95 °C for 2 min followed by 30 cycles of 45 s at 94 °C, 30 s at 57 °C, and 40 s at 74 °C, and with a final extension step at 74 °C for 5 min.

Table 1. Allele frequencies, observed heterozygosity (H_o), and polymorphism information content (PIC) for Omy0002DIAS in rainbow trout from four Danish hatcheries

	Allele frequency			
Allele size	Hatchery E	Hatchery H	Hatchery C	Hatchery D
174	0·20	–	0·02	–
176	0·11	0·26	0·13	0·16
178	–	–	0·02	0·06
180	0·05	0·01	0·11	–
182	–	0·01	0·02	0·18
186	–	–	0·14	–
190	0·13	0·12	–	–
202	–	–	0·05	–
206	–	–	0·07	0·02
208	0·20	0·12	0·07	0·04
212	–	–	–	0·02
222	0·05	0·06	–	0·10
224	0·09	0·24	0·30	0·25
236	0·05	0·03	–	–
238	0·07	–	–	0·02
240	0·02	–	0·05	0·07
242	0·04	–	–	–
246	–	–	–	0·02
248	–	0·12	0·02	0·07
254	–	0·03	–	–
H_o	0·71	0·74	0·86	0·82
PIC	0·85	0·80	0·83	0·84
n	28	39	28	34

Mendelian inheritance: Mendelian inheritance was confirmed by typing two halfsib families.

Polymorphism: Twenty alleles in the size range 174–254 bp were detected in samples from four hatcheries, as can be seen in Table 1. The allele sizes were determined by comparison to the PCR product from the sequenced clone and by utilization of internal size markers together with each sample.

Chromosomal location: Unknown.

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Assignment of the presenilin 2 gene (*PSEN2*) to bovine chromosome 16q16

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Sequence of PCR primers and PCR conditions: Primers were designed from the reported sequence (GenBank accession number U50871) of human *PSEN2*. ZOO–PCR was performed on bovine genomic DNA and the PCR product was sequenced to verify that the corresponding bovine locus had been amplified. PCR primers were

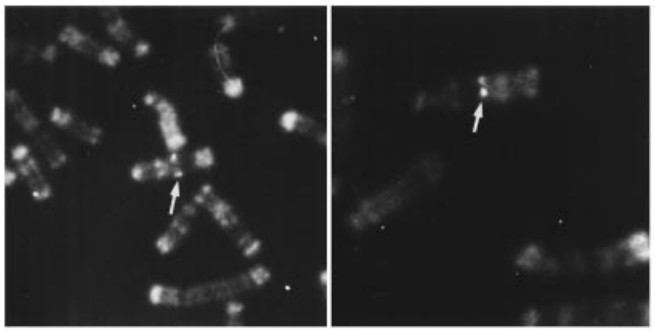

Fig. 1. R-banded metaphase spread after *in situ* hybridization with a YAC clone containing the bovine *PSEN2* gene. Arrows indicate signal on BTA16q16.

A.1: 5′ GATGGGAGGCCAGGGTGTC 3′ and A.2: 5′ CCTCCTGCT-GCTTGCTGTGTT 3′. A 128-bp fragment was amplified in 10 μl PCR containing 50 ng of genomic DNA and 1·5 mM $MgCl_2$. Samples were preheated for 5 min at 94 °C and subjected to 30 cycles at 94 °C for 30 s, 62 °C for 30 s, 72 °C for 30 s, and to a final extension step of 5 min at 72 °C in a Cetus 9600 thermocycler, using a Promega PCR kit (Promega, Madison, WI).

Description of the probe and details of FISH: The same primers were used to screen bovine YAC library[1] in order to isolate *PSEN2* containing YAC clones which were used for FISH experiments as described[2].

Chromosomal location: Two YAC clones were identified as containing the *PSEN2* bovine gene and one of these clones was used for FISH analysis. This YAC produced a strong signal on BTA16q16 and a secondary, faint and non-frequent signal on BTA9 (Fig. 1). To confirm the FISH result, somatic cell hybrid analysis[3] was performed by PCR and the gene was assigned to BTA16 with a correlation coefficient of 0·81[4]. *PSEN2* has been previously mapped to human chromosome 1q31-q42. This localization agrees with the updated comparative mapping data between man and cattle that show a correspondence between bovine chromosome 16 and two different regions of human chromosome 1, p36-ter and q23–42. More information on the gene order of both chromosomes is necessary to give a precise correspondence of chromosome segments between BTA16 and HSA1.

Acknowledgements: The authors thank C. Giraud-Delville and P. Laurent for technical assistance.

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The highly polymorphic canine microsatellite ZuBeCa6 is localized on canine chromosome 5q12–q13

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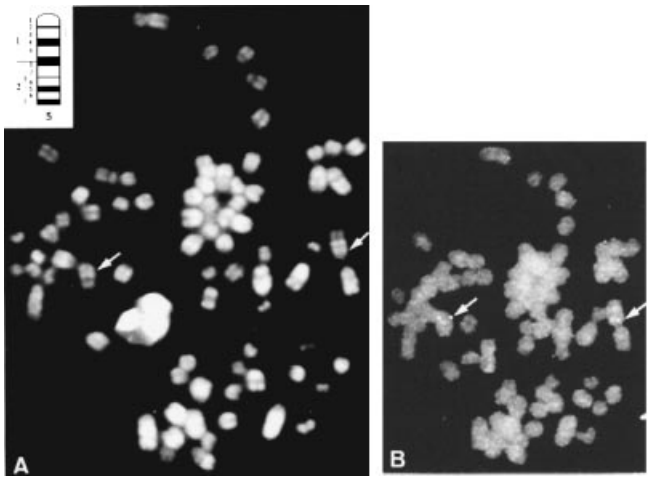

Fig. 1. QFQ-banded partial metaphase (A) prior to FISH (B) with the cosmid ZuBeCa6. Chromosome 5 (A) and hybridization signals (B) are indicated by arrows. Inset is a schematic representation of chromosome 5 adapted to the GTG banding system.

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Source/description: Positive clones were isolated from a genomic canine cosmid[1] library after screening with a mixture of the end-labelled oligonucleotide probes $(AAAG)_{20}$, $(GGAT)_{20}$ and $(GACA)_{20}$. After *Sau*3A subcloning into a pUC19 plasmid vector (Boehringer Mannheim, Mannheim, Germany) and rescreening a positive clone ZuBeCa6 was isolated and sequenced (EMBL accession number AJ224121). Primers flanking the sequence $(GAAA)_2$ GAGAGAG-CAGTGAGAGA $(GAAA)_1$ GAGAGAGA $(GAAA)_2$ G $(GAAA)_2$ GAAGA $(GAAA)_2$ GAA $(GAAA)_7$ GAAGA $(GAAA)_{11}$ $(GGAA)_{14}$ $(GAAA)_1$ GA $(GAAA)_{21}$ were designed using the OLIGO 5·0 program (National Biosciences, Plymouth, MN).

Primer sequences

5′ GCCATAAGCCCCAAGCCAGCAG 3′

5′ TGCCTCGTCAGCCCCTCTTTCC 3′

PCR conditions: Amplifications were carried out as previously described[1]. Thermocycling was performed using the following touch-down program[2]: initial denaturation for 3 min at 95 °C, two cycles each of 30 s at 95 °C, 30 s at 65 °C to 55 °C and 30 s at 72 °C, followed by 14 cycles of 30 s at 95 °C, 30 s at 55 °C and 30 s at 72 °C and 30 min final extension at 72 °C. Sizes of the alleles were determined on 8% denaturing polyacrylamide gels using a LI-COR DNA sequencer model 4000 L (LI-COR, Lincoln, NE).

Polymorphism: The sizes and frequencies of alleles observed in a panel of 52 dogs, representing 52 different breeds, are given in Table 1.

PIC: The calculated heterozygosity and PIC[3] were 0·95 and 0·95, respectively.

Chromosomal location: ZuBeCa6 was localized by FISH[4] to

Table 1. Sizes and frequencies of alleles observed

Allele (bp)	290	294	300	302	306	310	314	318	322
Frequency	0·038	0·019	0·019	0·105	0·086	0·019	0·067	0·010	0·029
Allele (bp)	325	327	329	330	333	334	336	340	341
Frequency	0·029	0·029	0·019	0·048	0·019	0·019	0·067	0·038	0·010
Allele (bp)	343	346	348	350	352	353	356	359	363
Frequency	0·038	0·048	0·010	0·056	0·010	0·010	0·048	0·010	0·029
Allele (bp)	365	367	369	376	383	387			
Frequency	0·010	0·019	0·010	0·010	0·010	0·010			

chromosome 5q12–q13 (Fig. 1) according to the partial canine standard karyotype[5].

Mendelian inheritance: Codominant inheritance was observed in five Beagle families with seven, seven, three, 13 and nine offspring, respectively.

Other comments: ZuBeCa6 is difficult to score due to the large number and wide range of allele sizes. ZuBeCa6 amplifies in wolf and fox.

Acknowledgements: We thank E. Garbely, U. Sattler and B. Colomb for their technical assistance and P. Schawalder for providing dog blood samples. This work was supported by the Albert-Heim Stiftung, Switzerland, and by the Union Bank of Switzerland acting on behalf of a client.

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A polymorphic mink (*Mustela vison*) dinucleotide repeat

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Source description: A mink cosmid library was constructed from a Royal Pastel male. The cosmid clone SH248 was isolated after screening with a γ^{32}PdATP-labelled $(GT)_9$ oligonucleotide. Sequencing of the microsatellite repeat was performed after subcloning of a *Sau*3A digested cosmid in the *Bam*H1 site of the pUC19 vector. The marker was named Mvi248.

Chromosomal localisation: The cosmid SH248 was localised to chromosome 10p-terminal by fluorescent *in situ* hybridisation.

PCR conditions: Approximately 40 ng of DNA was amplified in a total volume of 25 μl containing 15 pmol of each primer, 0·2 mM dNTP, 0·4 U *Taq* polymerase (Pharmacia) and 2·5 μl 10× PCR buffer (100 mM Tris–HCl, pH 9·0, 15 mM $MgCl_2$, 500 mM KCl, 0·1% v/w gelatine and 1% Triton X-100). Amplification was carried out in a OmniGene Temperature cycler (Hybaid). PCR cycling consisted of a denaturing step of 3 min at 93 °C, followed by 30 cycles of 52 °C for 20 s, 72 °C 10 s followed by 30 s at 93 °C, a final cycle at 72 °C for 20 min was performed.

Mendelian inheritance: Autosomal codominant segregation was demonstrated in a Danish fullsib mink pedigree.

Polymorphism: Studies for variation was performed in five populations of unrelated farm mink; two lines of Scanblack, two lines of Royal Pastel and one line of Standard mink 'Wild' (Table 1). The size of the alleles were scored using a 50–500 bp ladder (Pharmacia) together with the amplified isolated clone as an external size marker.

Table 1. Microsatellite loci from American mink

Marker	Primer sequence (5′-3′)	No. of Alleles	Product size (bp)	Heterozygosity	PIC	Annealing temperature	GenBank accession no.
Mvi248	GAATGGAGCACTTATTCAGAGG AAGAGACCCAATTAACACAGG	7	145	0·79	0·76	52	U87249

Acknowledgements: We thank Mr S. Svendsen for skilful technical assistance. This work was supported by the Danish Project AVL 93-SH-2.

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A *Pvu*II RFLP at the porcine orosomucoid (*ORM*) locus

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Probe description: A partial porcine orosomucoid (α_1-acid glycoprotein) cDNA[1] (EMBL/GenBank accession number M35990) in the recombinant plasmid pAGP-3 was excised using the restriction enzyme *Pst*I. The cDNA of 668 bp, after gel purification, was radiolabelled with [α^{32}]-dCTP and used to probe Southern blots.

Hybridization conditions: Hybridization conditions were as follows: 5× SSC, 0·5% SDS, 5% dextran sulphate, 5% PEG, 1% blocking reagent (Boehringer Mannheim, Milan, Italy); temperature 65 °C. First wash: 2× SSC, 0·1% SDS for 15 min at room temperature. Second wash: 0·2× SSC, 0·1% SDS for 10 min at 65 °C.

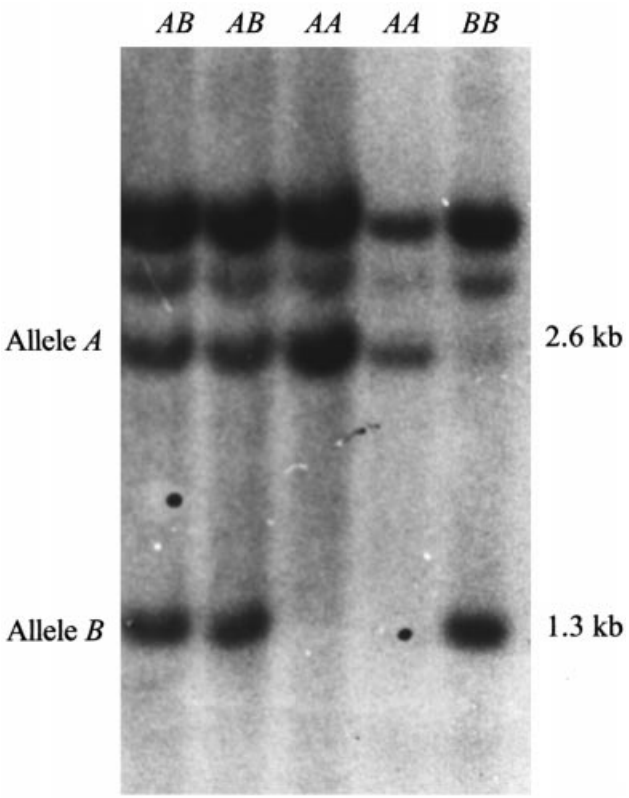

Fig. 1. Autoradiogram showing the *Pvu*II RFLP genotypes at the orosomucoid (*ORM*) locus in five pigs.

Polymorphisms: Two codominant alleles were observed with *Pvu*II. The allelic fragments revealed were ≈2·6 kb (allele *A*) and 1·3 kb (allele *B*) (Fig. 1). Additional polymorphisms were also detected with *Hin*cII and *Pst*I as previously reported[2,3].

Mendelian inheritance and allele frequency: The inheritance of the *Pvu*II alleles was confirmed in the Dutch PiGMaP reference pedigree[4]. Two-point linkage analysis was performed with CRI-MAP version 2·4[5] using the *Pvu*II RFLP and the ResPig database of the PiGMaP Consortium[4]. The results showed that *Pvu*II RFLP was linked to *Pst*I RFLP and *Hin*cII RFLP already described at the *ORM* locus[2,3] (θ = 0·00, LOD = 3·31 and θ = 0·00, LOD = 3·91, respectively). Allele frequencies were obtained in a sample of 45 unrelated pigs belonging to six different breeds (Large White no. 18: *A* = 0·08, *B* = 0·92; Piétrain no. 6: *A* = 0·17, *B* = 0·83; Duroc no. 5: *A* = 0·10, *B* = 0·90; Belgian Landrace no. 4: *A* = 0·25, *B* = 0·75; Landrace no. 4: *A* = 0·12, *B* = 0·88; Meishan no. 4: *A* = 1·00, *B* = 0·00).

Chromosomal localization: Chromosome 1[4,6].

Acknowledgements: We wish to thank Dr R. T. Stone (USDA, ARS, Roman L. Hruska US Meat Animal Research Center, Clay Center, NE, USA) who provided the cDNA probe. This study was funded by the Italian MURST 60% and was associated with the PiGMaP Consortium.

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Novel primers for the mitochondrial Control Region and its homologous nuclear pseudogene in the Eider duck *Somateria mollissima*

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Source/description: The highly polymorphic mtDNA Control Region is a common molecular genetic marker. In several bird species, a nuclear pseudogene homologous to the Control Region exists[1] which makes the specific amplification of the latter difficult. The primers GluL and H401 amplify the Control Region from mtDNA enriched isolates of Eider duck (from liver[2]; accession number AJ005266). From genomic DNA preparations of the same individual (from erythrocytes), however, the same primers amplify the nuclear pseudogene (accession number AJ005265). Novel forward primers were designed within the 'hairpin'[3] of Eider duck mtDNA and pseudogene sequences: From total DNA preparations, Lmtpin selectively amplifies the mtDNA Control Region, while Lncpin amplifies the homologous nuclear pseudogene.

Primer sequences:

forward primers:
GluL
5′-CACTACCCGGGACCTACAGCTCGAA-3′

Lmtpin
5′-CCATAGCACCGTACTTAACAACCCC-3′

Lncpin
5′-CTCATACCACCGTACTTAACCCCCT-3′

reverse primer:
H401
5′-GTGAGGTGTACGATCAATAAAT-3′

PCR and sequencing conditions: Genomic DNA (100–1000 ng) was used as a template. PCR was performed in a total volume of 75 μl, containing 10 mM Tris–HCl, pH 9·0, 50 mM KCl, 1·5 mM $MgCl_2$, 0·05 mM of each dNTP, 0·2 μM of the reverse and one of the forward primers, and 1·5 U *Taq* polymerase (Appligene, Illkirch, France). The reaction profile was as follows: one cycle 94 °C 5 min, 88 °C 1 min (*Taq* was added at this step); 40 cycles 94 °C 1 min 30 s, 55 °C (with Lmtpin, Lncpin) or 48·5 °C (with GluL) 1 min 15 s, 72 °C 1 min 30 s; one cycle 72 °C 2 min 30 s in a GeneAmp 2400 thermocycler (Perkin Elmer, Norwalk, CT). Reaction products were purified with the PCR Purification Kit (Qiagen, Hilden, Germany). Both strands were sequenced with the Thermo-Sequenase Dye Terminator Cycle Sequencing Kit (Amersham, Buckinghamshire, UK) and analysed on an ABI 373 automatic sequencer (Applied Biosystems, Foster City, CA).

Polymorphism: The nuclear pseudogene was monomorphic in 10 Eider Ducks of different origin (accession number AJ005265); 354 bp scored sequence of the mitochondrial Control Region of nine Eider specimens from three Baltic locations contained six polymorphic positions (all transitions), defining seven different haplotypes (accession number AJ005266–AJ005272) with an average pairwise nucleotide divergence of 0·73%. Haplotypic diversity[4] was 0·94.

Comments: The amplified part of the mitochondrial Control Region is a highly polymorphic maternal marker for the Eider duck. The described primers Lmtpin and H401 were also tested for the two main domestic duck species. With an annealing temperature of 53 °C, they successfully amplify the homologous mtDNA region in Muscovy duck (*Cairina moschata*; cf. accession number L16769) and a shorter fragment of the Control Region in Peking duck (*Anas platyrhynchos*; cf. accession number L16770)[5]. The Control Regions of Muscovy duck and Peking duck differ from that of the Eider duck at 32·3% and 27·7% of 300 compared nucleotides, respectively. The difference between the Control Region and its nuclear homologous pseudogene within the Eider duck was only 19·7%, suggesting a relatively recent origin of the pseudogene. Therefore, the identity of PCR amplified products (mtDNA or nuclear pseudogene) should be confirmed, when applying our primers to other Anatidae.

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Two polymorphic mink (*Mustela vison*) dinucleotide repeat loci

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Source description: A mink cosmid library was constructed from a Royal Pastel male. The cosmid clones SH389 and SH355 were isolated after screening with a γ^{32}PdATP-labelled $(GT)_9$ oligonucleotide. Sequencing of the microsatellite repeat was performed after subcloning of *Sau*3A digested cosmid in the *Bam*H1 site of pUC19 vector. The markers were named Mvi389 and Mvi355, respectively.

Chromosomal localisation: The cosmids SH389 and SH355 were localised by fluorescent *in situ* hybridisation to chromosome 8q1·2 and 12q1·2, respectively.

PCR conditions: Approximately 40 ng of DNA was amplified in a total volume of 25 μl containing 15 pmol of each primer, 0·2 mM

Table 1. Microsatellite loci from American mink

Marker ID	Primer sequence (5′-3′)	No. of alleles	Product size (bp)	Heterozygosity	PIC	Annealing temperature	GenBank accession no.
Mvi248	GCTCGTGTTTCTGGCTCT CCTGGGCTTTCCTGTTTAT	2	210	0·50	0·38	55	U87248
Mvi389	GAAGGCTAGGTAGGCATTAACC TTGTCCCATCCTTCACAGC	3	112	0·42	0·33	55	U87251

dNTP, 0·4 U *Taq* polymerase (Pharmacia) and 2·5 μl 10× PCR buffer (100 mM Tris–HCl, pH 9·0, 15 mM $MgCl_2$, 500 mM KCl, 0·1% v/w gelatine and 1% Triton X-100). Amplification was carried out in a OmniGene Temperature cycler (Hybaid). PCR cycling consisted of a denaturing step of 3 min at 93 °C, followed by 30 cycles of 52 °C for 20 s, 72 °C 10 s followed by 30 s at 93 °C, a final cycle at 72 °C for 20 min.

Mendelian inheritance: Autosomal codominant segregation was demonstrated in a Danish fullsib mink pedigree.

Polymorphism: Studies for variation were performed in five populations of unrelated farm mink; two lines of Scanblack, two lines of Royal Pastel and one line of Standard mink 'Wild' (Table 1). The size of the alleles were scored using a 50–500 bp ladder (Pharmacia) together with the amplified isolated clone as an external size marker.

Acknowledgements: We thank Mr S. Svendsen for skilful technical assistance. This work was supported by the Danish Project AVL 93-SH-2.

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Fourteen new polymorphic equine microsatellites

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Source/description: Equine genomic DNA was digested with *Sau*3AI; DNA fragments ranging from 300 to 800 bp in length were ligated into the *Bam*HI site of predigested Lambda Zap Express vector (Stratagene). The ligation reaction was packaged into phage particles with the Gigapack II Gold packing extract. Phage clones containing GT:AC dinucleotide repeats were identified by screening with a ^{32}P-labeled $(GT)_{10}$ probe. Inserts from positive phage were sequenced directly on an ABI-377 sequencer. Sequenced clones contained from 11 to 22 copies of the dinucleotide repeat. Primer pairs for PCR amplification of each microsatellite from genomic DNA were identified using the Prime program contained within the GCG Software Package (University of Wisconsin, WI).

PCR conditions: PCR reactions contained 12·5 ng DNA, supplied buffer (Boehringer), 1·5 mM $MgCl_2$, 0·5 μM each primer, 40 μM dCTP, dGTP, dTTP, 10 μM dATP, 12·5 nCi [α-^{32}P]dATP and 0·5 U *Taq* polymerase in a final volume of 10 μl. PCR amplification was

Table 1. Genotyping primers and conditions

Locus	Primer sequence (5′→3′)	Annealing temperature (°C)	[$MgCl_2$] (mM)	Size range (bp)	Alleles	No. of heterozygous stallions
UM026	CCCAAAATCAATTAGGTCTC ATCAGTTGCTCTCTACTTTTC	56	1·5	208–220	4	12
UM027	TGCAAGAATTGTGAGGGAC GTGCTCAGTTAGTGGTATTC	56	1·5	235–239	3	3
UM028	GCTCTGCCTCATTCTTATC TTGAGGACGGAGTGTTG	56	1·5	127–129	2	1
UM029	CTTTCATCTTTTGGCCCC CTGTCTTGCTTTCCCACC	56	1·5	177–185	3	7
UM030	CCGTGAAGTCACAGACTTAG ACAGTTTCTACAACAAACTGA	51	1·5	127–143	6	9
UM031	AGCTCAAACCAACCTTTC CCTTAAAACAGACACACTAC	56	1·5	136–148	5	9
UM033	CATTGTCCTGAGCAAGTC CTATCCGTCAAGTGTTTC	56	1·5	126–140	4	10
UM034	TTGAAACCCTTCAGACCC CAGAGAGCAAGTGTGAG	56	1·5	102–117	4	7
UM035	GTGATGGATGACATGAGG GCATTTAAAACACTAGAACAC	52	1·5	203–207	2	1
UM037	TCATTTTATCCTCCACCTC AAAAGGGCGTAATATGG	52	1·5	107–118	5	6
UM038	CAAGACAGAACAGAAGAAGAC ATATGGCTCGCTCCTAC	56	1·5	113–137	7	8
UM040	CTCTTGTACATGTCTCCTTGTC TACTTTCTCTCTTCCAAACC	51	1·5	250–270	6	9
UM042	GGCATCCCACATACAAAG GAAGCAACAGTCAATTCAG	56	1·5	218–226	4	8
UM043	CCTCAATCTTTTCTTCTCC TCAAGAGAGACGCTACAC	56	1·5	145–150	3	10

Table 1. Sequence and primer information for eight equine microsatellite loci

Locus (accession no.)	Repeat sequence	Primer sequences
NVHEQ26	$(GT)_{11}$	F: GGAAGTGGAACCAACTATGACA
(AF056392)		R: TGGAGGTTTAAGGGACGCTAT
NVHEQ29	$(TG)_{17}$	F: GAGATTTTGCCCCAAAGGTTA
(AF056393)		R: CTCTTCTTTCTTCCCCAGGTCT
NVHEQ31	$(CA)_{12}$	F: AACTCAAAAGCACAAGCCCACAC
(AF056394)		R: CAACACAGTGATGACCCAGGTAGG
NVHEQ40	$(CA)_3TG(CA)_{13}AG(CA)_2$	F: TGGCATCTGAATGGAGAATG
(AF056395)		R: GATTATGATGCTACAGGGGAAAG
NVHEQ43	$(CCA)_5(CA)_{22}$	F: TGACACAAGATAAAAGCCCCAGG
(AF056396)		R: GATTGGGAAAAGAGCACAGCC
NVHEQ90	$(GT)_{13}$	F: TCTATGTGTCAGAATGAGGAGGT
(AF056397)		R: TTTTGCAAGGTAGATAGATGCC
NVHEQ98	$(CA)_{10}$	F: TGCAATGACCATCAGAACTTC
(AF056398)		R: CGGTGGTTTTGGAAATGAA
NVHEQ100	$(TG)_{22}T(TG)_2$	F: CCAAAGCAGAACATGTGAAGTT
(AF056399)		R: TGGCATAGATGTTAGCTCAGTGA

F, forward; R, reverse.

performed in 96-well plates for 30 cycles with initial denaturation at 94 °C for 30 s and the following temperature cycles were carried out: annealing at 51–56 °C for 30 s, extension at 72 °C for 30 s. The amplification was carried out using a PTC 100 thermal cycler (MJ Instruments). Electrophoresis was performed on BioRad Sequi-Gen® 38 × 50 cm plate sequencing gel units.

Polymorphism: DNA from the 13 reference family stallions of diverse breeds from the International Equine Genome Mapping Workshop was amplified and genotypes were determined by autoradiography. In this sample population, the newly identified equine microsatellites exhibited 2–7 alleles with 1–12 of the stallions showing heterozygosity (Table 1).

Chromosomal locations: Unknown.

Acknowledgements: We are grateful to the International Equine Genome Mapping Workshop for providing the reference family stallion DNA. This work was supported in part by a Grant in Aid from the University of Minnesota Graduate School and a grant from the North American Equine Ranching Information Council.

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Eight new equine dinucleotide repeat microsatellites at the *NVHEQ26*, *NVHEQ29*, *NVHEQ31*, *NVHEQ40*, *NVHEQ43*, *NVHEQ90*, *NVHEQ98* and *NVHEQ100* loci

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Source/description: Equine genomic DNA was digested with *Sau*3AI and size selected fragments (300–600 bp) were ligated into the *Bam*HI site of the BluescriptSK + plasmid. The library was screened with a synthetic $(GT)_{10}$ oligonucleotide end-labeled with $\gamma^{32}P$ ATP. Positive clones were picked and rescreened and the double positives were sequenced with dye terminator chemistry on an ABI 310 sequencer. The sequences have been submitted to the GenBank database (accession numbers are listed in Table 1). Primers for the PCR were designed to amplify the regions containing the dinucleotide repeats (Table 1).

PCR conditions: The forward primers were end-labeled with $\gamma^{33}P$ ATP. PCR amplifications were performed on a GeneAmp PCR System 9600 (Applied Biosystems, CA) thermal cycler in 10 μl reaction mixtures containing: 20–40 ng of genomic template DNA, 2 pmol of each primer, 50 mM KCl, 1·5 mM $MgCl_2$, 10 mM Tris–HCl, 0·2 mM dNTP and 0·5 U AmpliTaq. Thermocycling parameters after denaturation at 94 °C for 5 min were 28 cycles of: 95 °C for 1 min, 60 °C for 30 s and 72 °C for 1 min followed by 10 min at 72 °C. The PCR products were run on a denaturing 6% polyacrylamide gel (7·5 M urea) and visualized by exposure of the gel to X-ray film.

Polymorphism: Amplification products were tested for polymorphism in a panel of 30–34 unrelated animals representing Thoroughbred, Standardbred, Norwegian Trotter and Norwegian Fjord. Alleles were named according to their sizes as determined by comparison to an M13 sequence ladder. Estimated heterozygosity was calculated using the observed allele frequencies and assuming no null alleles (Table 2). Allele sizes for the first four horses of the 1994 ISAG/ISBC standardization test at each of the eight microsatellite loci are shown in Table 3.

Table 2. Polymorphism of equine microsatellite loci

Locus	Alleles (*n*)	Size range of products (bp)	Size of M-allele[a]	Heterozygosity
NVHEQ26	2	140–142	140	0·36
NVHEQ29	6	91–113	101	0·77
NVHEQ31	2	128–130	128	0·33
NVHEQ40	6	144–158	152	0·68
NVHEQ43	7	135–159	151	0·78
NVHEQ90	3	99–105	101	0·35
NVHEQ98	2	167–169	167	0·33
NVHEQ100	8	185–207	197	0·74

[a]For alphabetic nomenclature.

Table 3. Allele sizes for the first four horses of the 1994 ISAG/ISBC standardization test

Horse	1	2	3	4
NVHEQ26	140/142	140/142	140	142
NVHEQ29	101	107	101	101
NVHEQ31	128/130	128/130	130	130
NVHEQ40	154/156	154	154	154
NVHEQ43	141/145	141/145	153	151/153
NVHEQ90	101	101	101	101
NVHEQ98	167	167/169	167	167
NVHEQ100	207	207	207	191/207

Chromosomal locations: Unknown.

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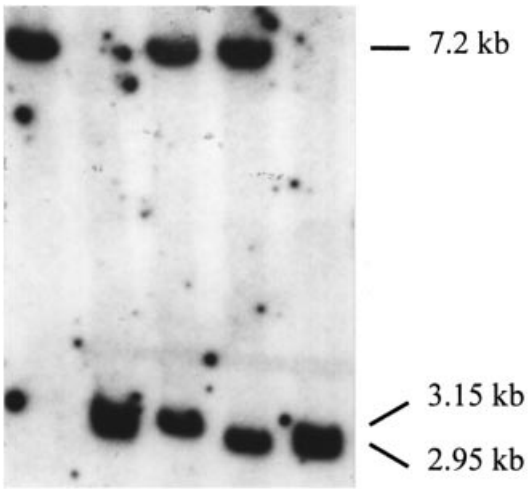

Fig. 1. The *Xba*I RFLP at the *FGA* locus. Genotypes from left to right are: *A1/A1*, *A2/A3*, *A1/A2*, *A1/A3*, *A2/A3*. Alleles: *A1* = 7·2 kb, *A2* = 3·15 kb and *A3* = 2·95 kb.

RFLP and linkage analysis of the porcine fibrinogen loci – *FGA, FGB* and *FGG*

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Probes: Porcine cDNAs encoding α-fibrinogen (015–3B; EMBL accession number: M29514), β-fibrinogen (020–6FL; EMBL accession number: M29513) and γ-fibrinogen (019–2D; EMBL accession number: M29510) were isolated previously as pBluescript clones from a liver cDNA library established in λZAPII[1]. The EMBL database was searched (19th April 1998) with partial DNA sequences of these cDNA clones. The putative α-fibrinogen sequence was 97·2% identical to a pig DNA sequence (EMBL: D43760) shown previously to be homologous to α-fibrinogen sequences from humans, rhesus monkey, dog, rat, mouse and Syrian hamster[2]. The putative porcine β-fibrinogen sequence was 83·7% and 81·3% identical to sequences from exons 1 and 2 of the human β-fibrinogen gene, respectively (EMBL: J00130 and J00131). The putative porcine γ-fibrinogen sequence matched γ-fibrinogen sequences from humans (EMBL: X51473, 82% identity; M10014, 71% identity), European polecat (EMBL: U28494, 77% identity), cattle (EMBL: X15556, 81% identity), and rat (EMBL: J00734, 70% identity). We conclude that the cDNA clones 015–3B, 020–6FL and 019–2D represent the porcine homologues of α-fibrinogen (*FGA*), β-fibrinogen (*FGB*) and γ-fibrinogen (*FGG*), respectively.

Methods: Restriction enzyme digests, agarose gel electrophoresis,

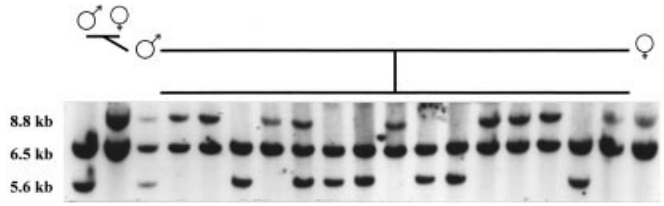

Fig. 2. The *Eco*RI RFLP at the *FGB* locus. Alleles: *B1* = 8·8 kb + 6·5 kb; and *B2* = 6·5 kb + 5·6 kb and *B3* = 6·5 kb. Genotypes from left to right are *B2/B2*, *B1/B1*, *B1/B2*, *B1/B1*, *B1/B1*, *B2/B3*, *B1/B3*, *B1/B2*, *B2/B3*, *B2/B3*, *B1/B3* *B2/B3*, *B2/B3*, *B1/B1*, *B1/B1*, *B1/B1*, *B2/B3*, *B1/B3*, *B1/B3*. The relative intensities of the fragments were used to determine whether the *B3* allele was present or not.

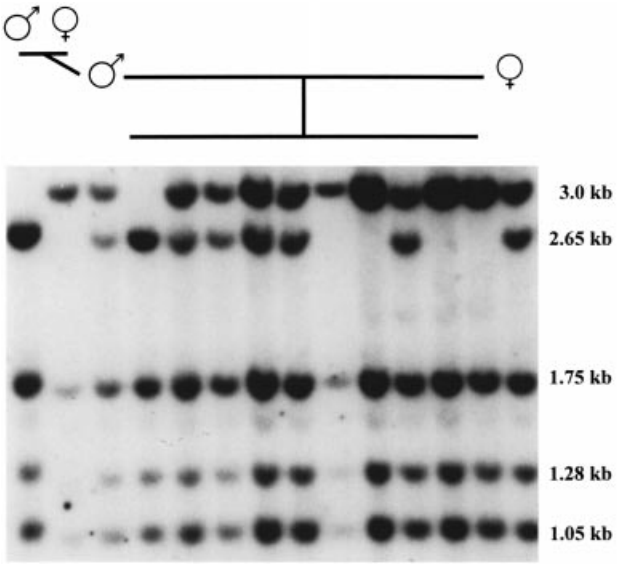

Fig. 3. The *Taq*I RFLP at the *FGG* locus. Alleles: *C1* = 3·0 kb and *C2* = 2·65 kb.

Southern blotting, hybridizations and autoradiography were performed as described previously[3].

Polymorphisms: DNA samples from eight pigs (two of each sex from both the Chinese Meishan and British Large White breeds) were screened for polymorphisms with a panel of 10 restriction endonucleases (*Bam*HI, *Eco*RI, *Eco*RV, *Hin*dIII, *Kpn*I, *Msp*I, *Pst*I, *Sac* I, *Taq*I, and *Xba*I) in combination with the fibrinogen cDNA probes. These eight pigs represent the grandparents of the Scottish PiGMaP reference families[4]. Polymorphic fragments were found with the following combinations of restriction enzymes and probes – α-fibrinogen (*Bam*HI, *Kpn*I, *Pst*I, *Sac*I, *Taq*I, *Xba*I); β-fibrinogen (*Eco*RI, *Msp*I, *Taq*I, *Pst*I); and γ-fibrinogen (*Bam*HI, *Hin*dIII, *Kpn*I, *Msp*I, *Pst*I, *Sac*I and *Taq*I). The α-fibrinogen probe revealed three allelic fragments with *Xba*I: 7·2 kb, 3·15 kb and 2·95 kb (Fig. 1). The 8·8 kb, 6·5 kb and 5·6 kb fragments revealed with the β-fibrinogen probe in *Eco*RI digested DNA (Fig. 2) could be attributed to three allelic combinations: 8·8 kb + 6·5 kb (*B1*); 6·5 kb + 5·6 kb (*B2*) and 6·5 kb (*B3*). Two allelic fragments of 3·0 kb and 2·65 kb were found in *Taq*I digests hybridized with the γ-fibrinogen probe as well as three constant fragments of 1·75 kb, 1·28 kb and 1·05 kb (Fig. 3). The α-fibrinogen (*Xba*I), β-fibrinogen (*Eco*RI) and γ-fibrinogen (*Taq*I) RFLPs were shown to under simple autosomal monogenic control by segregation analysis of up to seven three-generation families with a total of 73 F_2 offspring. Interpretation of the inheritance patterns for the β-fibrinogen (*Eco*RI) RFLP was difficult as the 6·5 kb fragment behaved not only as an allele on its own but also as a component of the other two alleles.

Linkage analysis and mapping: No recombinant haplotypes were observed amongst 40 F_2 offspring from intercross matings between F_1 individuals heterozygous at all three RFLP loci described here. Thus, the porcine *FGA*, *FGB* and *FGG* are tightly linked. These fibrinogen loci have been mapped previously to chromosome 8 by linkage analysis[4]. The tight linkage observed amongst the porcine fibrinogen loci had been expected as the human *FGA*, *FGB* and *FGG* loci are found together in a 50-kb region on chromosome 4q23–q32[5]. The synteny of *FGA*, *FGB*, *FGG*, *ALB*, *IL2*, *PDGFRA* and *SPP1* is conserved in humans and pigs[4] and provides further support that these RFLPs map to the porcine homologues of *FGA*, *FGB* and *FGG*.

Acknowledgements: This study was conducted in the context of the European Pig Gene Mapping Project (PiGMaP) which is supported at the Roslin Institute with funds from the European Commission, the Biotechnology and Biological Sciences Council and the Ministry of Agriculture, Fisheries and Food.

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IDVGA75 (*D15S24*) a polymorphic microsatellite locus physically mapped to bovine chromosome BTA 15q25

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Source/description: A perfect (AC)17 repeat microsatellite, IDVGA75 (EMBL accession number: X89117; D number *D15S24*) was isolated by screening a partial cosmid library of *Sau*3AI digested bovine DNA in the vector SuperCos 1 with a labeled (TG)8 probe. The microsatellite repeat region was sequenced directly from the cosmid[1]. Primers were designed from the sequence flanking the microsatellite repeat using the OLIGO program (National Biosciences, Plymouth, MN). Oligonucleotides primers without self-complementary and with a *T*m between 60 and 65 °C were selected.

PCR conditions: PCR was carried out in a volume of 10 µl, containing 50 ng genomic DNA, 1 mM of each primer (pAG5 U2 (TG strand): 5′GGGGTCTCAAAGAGTCAGATAC3′ and pAG5 (AC strand): 5′TTTCTGGAGAACTTTGGCTATG3′), 200 mM dNTP, 1 mM MgCl2 and 1 U *Taq* DNA polymerase (Life Technologies, Paisley, UK). The pAG5 (AC strand) primer was labeled with [γ-^{33}P]ATP (Amersham International, Little Chalfont, Buckinghamshire, UK) by T4 polynucleotide kinase (New England Biolabs, Beverly, MA). DNA amplification was performed on a PTC-100 thermal cycler (MJ, Watertown, MA) using the following conditions: five initial cycles of denaturation at 94 °C (30 s), annealing at 60 °C (30 s) and elongation at 72 °C (45 s) followed by 30 additional cycles with the annealing temperature reduced to 55 °C. Finally an elongation step at 72 °C (3 min) was performed.

Allele size and frequency: Alleles were scored in a panel of 25 unrelated animals of five Italian and one Chinese breeds (Chianina, Frisona, Maremmana, Piemontese Vladostana and Nanyan). PCR products were separated on denaturing polyacrylamide-urea (7%-7 M) gels with a sequencing ladder and the microsatellite allele amplified from the parent cosmid clone (188 bp) as size markers. Seven alleles were observed with the corresponding allele frequency indicated in parentheses: 180 bp (0·28), 182 bp (0·20), 184 bp (0·16), 186 bp (0·08), 188 bp (0·16), 190 bp (0·10) and 192 bp (0·02).

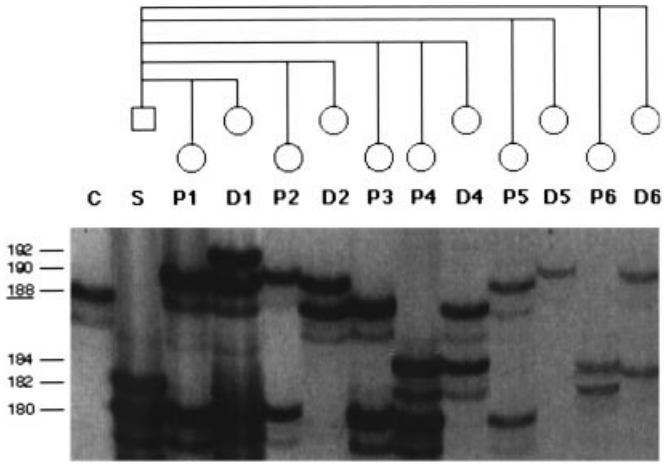

Fig. 1. Amplification products of microsatellite IDVGA75 in a halfsib family of a Piemontese sire crossed to five Nanyan dams. C, Cloned allele from cosmid; S, Piemontese sire; D1, D2, D4–D6, Nanyan dams; P1–P6, progeny. The size of alleles are shown to the left, with the cloned allele underlined.

Mendelian inheritance: A halfsib family composed of a Piemontese sire, five Nanyan dams and six progeny were typed for this microsatellite marker in order to confirm the Mendelian inheritance of the alleles; six alleles are shown to segregate in this family material (Fig. 1).

Chromosome location: The cosmid clone from which the IDVGA75 microsatellite was derived was localized to Q-banded metaphase chromosomes by fluorescence *in situ* hybridization (FISH) as described elsewhere[2]. A specific signal was identified on BTA 15q25.

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Genotyping of α-s1 casein in sheep

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Description: Four phenotypic α-s1 casein variants (*A*, *B*, *C* and *D* or *Welsh*) have been described in sheep. The amino acid (aa) sequences have been established for the *A*, *C*, and *D* variants[1]. The *C* variant differs from *A* by having a Pro instead of a Ser at position 13. The *D* variant is characterized by a further substitution at the position 68 where a SerP is replaced by an Asn. A DNA mutation characteristic for the *A* allele was detected by DNA digestion with the restriction enzyme *Mbo*II; as the mutation eliminates this restriction site in the third exon. Conversely, the restriction enzyme sites and subsequent digestion patterns are not altered by the mutation responsible for the aa change in the *D* variant (exon 9) sequence. However, when using a mismatching primer[2], a polymorphic additional *Mae*III site was introduced in the *nonD* alleles.

PCR conditions: For the detection of the allele *A* a 372-bp DNA fragment including the polymorphic exon III sequence was amplified using 50 pmol of the following primers A1: 5′GGTGTCAAATTTAGCTGTTAAA3′ and A2: 5′GCCCTCTTCTCT-AAAAAGGTTT3′ for 30 cycles. The annealing temperature and time were 53 °C and 30 s, respectively; 1·5 mM $MgCl_2$ final concentration, 200 mM each dNTP and ≈100 ng of genomic DNA. The detection of a 237-bp fragment including the polymorphic exon 9 for the *D* allele was amplified using the same conditions as stated above differing only in primers D1: 5′CAACATATTTTAAA-TAAATTGACAAT3′ and D2: 5′AATTAACATAAAAATGGCA-TACGTC3′. Digestions of the PCR amplified DNA fragments were performed according to the manufacturer's instructions.

Results: Amplification of the genomic region including exon 3 gave a DNA fragment of the expected length (372 bp). Regardless of genotype a 66-bp fragment after enzyme digestion was always present due to a common *Mbo*II site. An additional *Mbo*II site only occurred within the A allele. With respect to genotype (*nonA* homozygous, *A/A* individuals and heterozygous *nonA/A*), three different restriction enzyme patterns are observed: (1) two DNA bands (306 and 66 bp); (2) three DNA bands (160, 146 and 66 bp); (3)

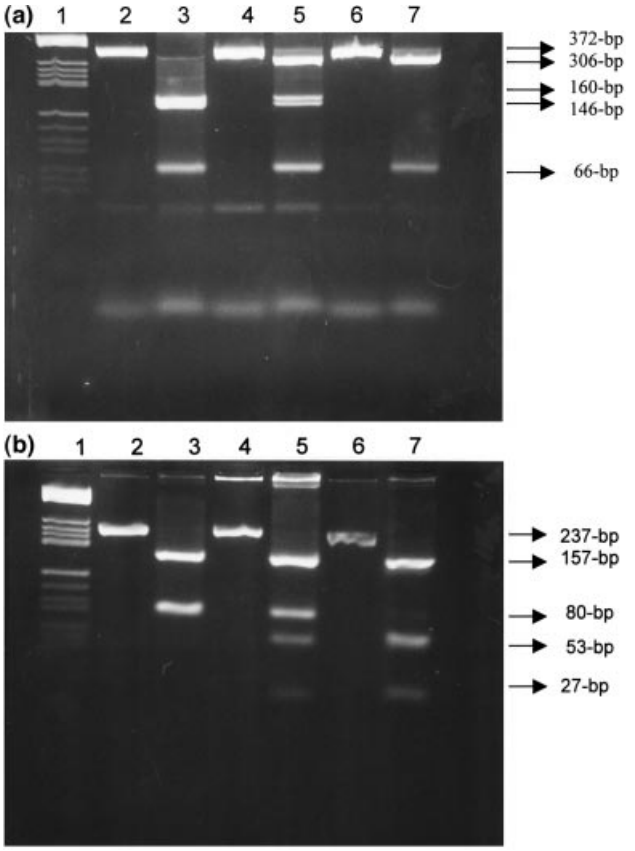

Fig. 1. (a) Genotyping at the ovine α-s1 casein locus, using PCR followed by *Mbo*II digestion. Agarose gel (2% Metaphore + 2% agarose) electrophoresis analysis of DNA fragments amplified with the primer pair A1 and A2 from homozygous *A*/*A* (lanes 2 and 3), heterozygous nonA/A (lanes 4 and 5) and 'homozygous' *nonA*/*nonA* (lanes 6 and 7). Lanes 2, 4 and 6 show undigested PCR products, whereas lanes 3, 5 and 7 correspond to the same fragments digested with *Mbo*II. Lane 1, molecular weight marker (pBR322, *Hae*III digested). Sizes (in bp) of DNA fragments are given on the right. (b) Genotyping at the ovine α-s1 casein locus, using PCR followed by *Mae*III digestion. Agarose gel (4:2% Metaphore agarose + 2% agarose) electrophoresis analysis of DNA fragments amplified with the primer pair D1 and D2 from homozygous *D/D* (lanes 2 and 3), heterozygous nonD/D (lanes 4 and 5) and 'homozygous' *nonD/nonD* (lanes 6 and 7). Lanes 2, 4 and 6 show undigested PCR products, whereas lanes 3, 5 and 7 correspond to the same fragments digested with *Mae*III. Lane 1, molecular weight marker (pBR3222, *Hae*III digested). Sizes (in bp) of DNA fragments are given on the right.

four DNA bands (306, 160, 146 and 66 bp), respectively (Fig. 1a). The D allele is detected since primer D2 is not an exact match for the DNA template with respect to a C replacing a G at position 23. This substitution creates a new *Mae*III site (position 210), while leaving a common *Mae*III site (position 157) unchanged in amplified DNA fragments from *nonD* individuals. Fragments yielded after digestion with *Mae*III of the 237-bp amplified DNA allowed us to distinguish the *D* allele (Fig. 1b). Homozygous individuals of *D/D* genotype give two fragments (157 and 80 bp). The heterozygous *nonD/D* show restriction pattern with four fragments (157, 80, 53 and 27 bp). Likewise, nonD/nonD present a three-band pattern (157, 53 and 27 bp). The DNA analysis agreed with the phenotype determined by IEF starting from milk of 50 individuals.

Comments: Genetic variants of milk protein can be related to the quantity and quality of milk production, observed for the ovine α-s1 casein *D* allele[3]. The aim of the present study was to develop a method to discriminate between the *A*, *C* and *D* alleles at DNA level. This method could be employed to eliminate from the breeding scheme males and non-lactating females carrying the unfavourable *D* allele as well as, facilitate the detection of polymorphisms occurring in the population for basic studies.

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PCR-RFLP test for direct determination of β-lactoglobulin genotype in sheep

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Description: Sheep β-lactoglobulin (β-LG) genotypes can be determined by milk protein analysis[1] (Fig. 1). The protein sequences of β-LG A and B variants differ for only one amino acid substitution: 38 Tyr (A)→His (B). The causal nucleotide mutation (T→C) also disrupts a *Rsa*I site and can be detected by PCR-RFLP analysis. A 120-bp PCR product surrounding the nucleotide mutation (exon 2) was amplified from genomic DNA with primers LBG1 and LBG2 and the PCR product was digested with *Rsa*I. Digestion of β-LG A allele yields three bands of 66, 37 and 17-bp in comparison to the β-LG B allele which gives only two fragments of 103 and 17-bp. The 17-bp fragment results from a *Rsa*I site present in both alleles and is useful as control of digestion (Fig. 2).

Primer sequences:

forward (LBG1): 5′-CAACTCAAGGTCCCTCTCCA-3′

reverse (LBG2): 5′-CTTCAGCTCCTCCACGTACA-3′

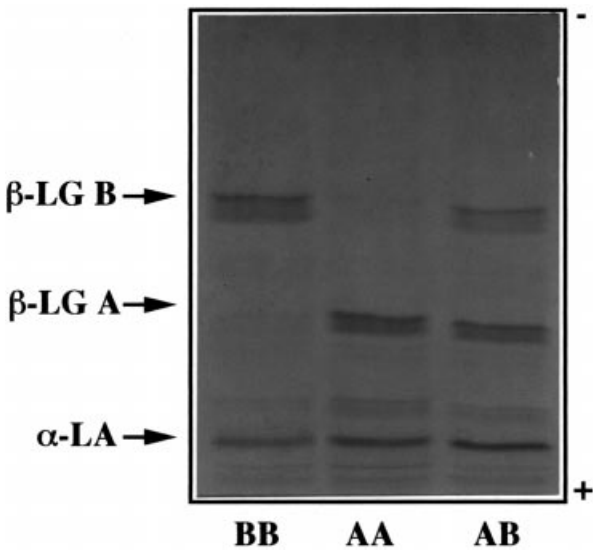

Fig. 1. IEF analysis of individual whey samples at β-LG locus; the three genotypes *AA*, *AB* and *BB* are reported.

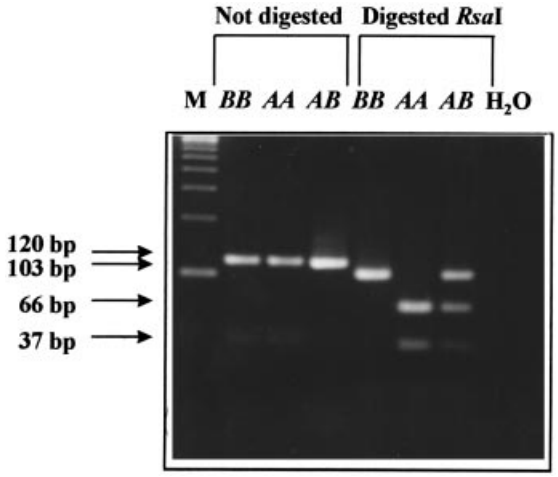

Fig. 2. Result of PCR and *Rsa*I digestion. The three genotypes *AA*, *AB* and *BB* are reported. The DNA correspond at the same animals analysed in Fig. 1. M, DNA marker 100 ladder (Amersham Pharmacia Biotech, Monza, Italy).

PCR and digestion conditions: The 50 μl reaction mix comprised of 100 ng genomic DNA, 150 nM of each primer, 1·25 U *Taq* Gold DNA polymerase (Perkin Elmer, Monza, Italy), 50 mM KCl, 10 mM Tris–HCl (pH 8·3), 2·5 mM $MgCl_2$, dNTPs each at 200 μM and 0·01% gelatin. Amplification was carried out using a 9600 DNA Thermal Cycler (Perkin Elmer). After a denaturation of 10 min at 95 °C the temperature cycling was as follows: 35 cycles of 15 s at 93 °C, 15 s at 60 °C, 30 s at 72 °C and a final extension of 10 min at 72 °C. Ten microlitres of the reaction was phenol/chloroform purified, precipitated and digested with 1 U of *Rsa*I (Boehringer Mannheim, Milano, Italy) in a final volume of 15 μl for 1 h at 37 °C. The result was directly analysed by electrophoresis in 3% agarose gel stained with ethidium bromide, followed by ultraviolet exposition.

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Thirteen bovine microsatellite markers that are polymorphic in sheep

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Source/description: We analysed 29 previously published bovine microsatellites on 25 sheep of the Dutch Texel breed. Animals were tested in pools of four or five; it was checked that this allowed the detection of all individual alleles. Markers were assigned a rank to represent the ease of interpretation as previously described[1]. (Rank 1, clear bands for each allele; rank 2, stutter bands which did not interfere with the scoring; and rank 3, severe stutter but alleles could still be identified.)

PCR conditions: Genomic DNA (50 ng) was amplified in 10 μl containing 10 pmol of ^{32}P-labelled left primer, 10 pmol of right primer, 0·2 mM dNTPs, 1·5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris–HCl pH 8·3, 0·001% gelatin and 0·2 U *Taq* polymerase (Promega, Madison, WI). After 2 min at 93 °C, 25 cycles of 15 s at 92 °C, 45 s at the indicated annealing temperature (Table 1) and 45 s at 72 °C were performed. The final step was 2 min at 72 °C.

Analysis of PCR generated fragments: PCR products were separated on a denaturing polyacrylamide gel (7%) and amplified fragments were visualised by autoradiography. Apparent allele sizes were determined by comparison with a M13 sequencing ladder.

Chromosomal location: The homologous karyotype of cattle and sheep chromosomes and extensive genome conservation between these species allows tentative assignments of the markers to sheep chromosomes[3,16] (Table 1).

Polymorphism: Seventeen bovine microsatellite primers amplified specifically in sheep and 13 were polymorphic with allele numbers ranging from 2 to 11 (Table 1). One of these markers (CSSM22) was previously reported to be monomorphic in sheep[16]. Four markers were monomorphic (IOBT730, IOBT918, IDVGA80, and HEL9) and 12 either did not amplify or gave multiple products (IOBT930, IOBT959, IOBT313, IOBT528, INRA209, CSSM42, CSSM14, HAUT24, HAUT37, HAUT29, HAUT27, and RM28). A similar succesrate of bovine primers in sheep has been described previously[2,16].

Acknowledgements: We thank P. Vellema, M.F. de Jong, J.

Table 1. Polymorphic markers

Bos taurus					*Ovis aries*				
Name	Chr.	Physical location	Acc. no.	Ref.	Alleles	Size	Chr.[a]	T_a[b]	Rank
IDVGA 37	2	2q45	X85053	4	6	188–200	2	55	3
HAUT 31	3	3q36	X89256	5	4	196–220	1	60	1
RM 188	4		–	6	11	118–144	4	55	3
CSSM 22	5		–	7	5	218–236	3	55	2
IOBT 450	6		X97564	8	2	126–130	6	55	1
AFR 227	6		X83436	9	4	96–110	6	60	1
IOBT 323	12	12q24	X89206	10	2	192–194	10	60	2
IOBT 395	15	15q27–28	X95067	11	5	90–120	15	60	1
IOBT 965	19	19q17–21	X93163	12	5	102–128	11	60	2
IOBT 1479	23	23q24–25	U59512	13	2	104–108	20	55	2
IOBT 1401	24	24q21–24	X95069	11	3	124–146	23	55	2
INRA 206	25	25q13	–	14	2	152–154	21	55	3
IOZARA1489	X	xq42–43	U47615	15	2	284–286	X	55	1

[a]Tentative assignment based on homology between cattle and sheep chromosomes[3,16].
[b]Annealing temperature (°C).
Chr., chromosome; Acc. no., accession number.

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AIRS-PCR for the canine DLA-DQA1 F141L polymorphism

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Source/description: A sequence variation that distinguishes several DLA (dog lymphocyte antigen)-DQA1 alleles occurs as a phenylalanine-leucine interchange at amino acid position 141 (F141L; e.g. GenBank accession numbers: M74908, M74910)[1]. A PCR-based test was developed to distinguish the underlying nucleotide difference by the artificial introduction of a restriction site (AIRS). The two alleles can be distinguished by digestion of the PCR product with *Bsl*I. The PCR product is 177 bp and the F allele is not cut. The L allele is cut into 157 and 20 bp fragments.

Primer sequences:

DLA-DQA1-F141L-D: GTTCCTGAGGTGACTGTGTT

DLA-DQA1-F141L-U: GAAGGAATGATCCCCC*T*TGG

The altered nucleotide that creates the *Bsl*I site with the L allele is in italics.

PCR/RSP conditions: Reaction components were: 10 mM Tris–HCl (pH 8·3 at 20 °C), 50 mM KCl, 2 mM $MgCl_2$, 80 μM dNTPs, 0·13 pmol of each primer, and 0·5 U *Taq* DNA polymerase. Target DNA was derived from blood or cheek swab DNA (50–200 ng). PCRs were performed in a final volume of 25 μl with the following cycle times: 94 °C, 4 min (1 cycle); 94 °C, 40 s, 63 °C, 1 min, 72 °C, 1 min (50 cycles). After PCR, 3 μl of 50 mM $MgCl_2$ and 10 U of *Bsl*I were added directly to the PCR mix and the reaction was incubated at 55 °C for at least 1 h. Eight microlitres of digested product were run on a 2% agarose (1× TBE) gel.

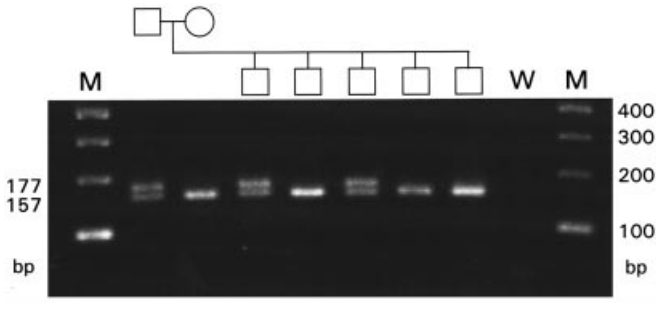

Fig. 1. Co-dominant Mendelian inheritance in a seven-member German shepherd dog pedigree. M indicates 100 bp ladder lanes and W indicates a water blank lane.

Allele frequencies: The frequency of each allele was examined in 10 Shetland sheepdogs, 10 Doberman pinschers, nine Cairn terriers, 14 American Cocker spaniels, 16 Beagles, and seven German shepherd dogs. The animals for the last two breeds are the parents of the DogMap panel[2]. The respective F and L allele frequencies (and heterozygosities) for the six breeds were: 0·65, 0·35 (0·4); 0·3, 0·7 (0·4); 0·72, 0·28 (0·11); 0·64, 0·36 (0·29); 0·78, 0·22 (0·44); and 0·79, 0·21 (0·14). Values for the combined data are: 0·65, 0·35 (0·32).

Mendelian inheritance: Co-dominant Mendelian inheritance was seen in a seven-member German shepherd dog pedigree (Fig. 1).

Chromosomal location: The *DLA-DQA1* gene, located within the major histocompatibility complex, is found on chromosome 12qter[3]. The human gene is found on chromosome 6p21·3.

Acknowledgements: This work was supported by the American Shetland Sheepdog Association.

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Mapping of the *ME1* locus to chicken chromosome 3

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Source/description: The chicken *ME1* gene (Malic Enzyme 1) has been partially cloned and sequenced[1]. From the published sequence (EMBL accession number U49693), we designed two PCR primers to amplify a promoter fragment of this gene. The expected length of the fragment was 177 bp.

Primer sequences:

MAL1U: 5′-CCG ATC CCC TCC AGA GAA GG-3′

MAL1L: 5′-TGC TGC GTG CGG TAC TGC CG-3′

PCR conditions: PCR amplifications were performed in 25 μl on a Techne PHC-3 thermocycler. The reaction mixes comprised 20 ng genomic DNA, 200 μM each dNTP, 1 μM each primer, 1·5 mM $MgCl_2$, 20 mM Tris–HCl (pH 8·4), 50 mM KCl and 1 U *Taq* polymerase (Gibco BRL, Rockfield, MD). The profile for thermal cycling was, for 30 cycles: denaturation 94 °C, 30 s; annealing 65 °C, 30 s; elongation 72 °C, 30 s. The amplified fragment was denatured by heating at 95 °C and loaded onto a non-denaturing acrylamide/bisacrylamide (49:1) gel containing 5% glycerol, to visualize a Single Strand Conformation Polymorphism[2] through silver staining[3].

Polymorphism and Mendelian inheritance: Two alleles were observed in the 177 bp fragment. Segregation analyses were performed in the East Lansing[4] chicken reference back-cross mapping population using the Map Manager software[5].

Linkage analysis and chromosomal location: The segregation pattern of *ME1* alleles in the reference population showed that this locus is located on chromosome 3, between ADL024 (5·8 cM, LOD 10·7) and ADL015 (9·6 cM, LOD 8·5). The homologous *Mod1* gene has been localized on mouse chromosome 9 and the human *ME1* one on chromosome 6q12[6]. This result extends the synteny conservation between a fragment of chicken chromosome 3 and HSA 6 to the proximal part of the q arm of this chromosome. Some

rearrangements have occurred, since *ME1* is localized on chicken chromosome 3 between *TCP1* and *PLN*, located on human 6q26–q27 and 6q22, respectively. Concerning the conservation of this genomic segment in the mouse genome, only one other gene of this region is localized on mouse chromosome 9, i.e. *GSTA2*.

Acknowledgements: We would like to thank L.B. Crittenden and Hans Cheng for providing DNA samples of the East Lansing chicken reference family.

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Characterisation and mapping of a highly polymorphic microsatellite within the porcine skeletal muscle triadin gene

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Source/description: Triadin is an intrinsic membrane protein first identified in the skeletal muscle junctional sarcoplasmic reticulum. It is considered to play an important role in excitation contraction coupling. However, structural analysis indicates that the triadin is co-localised with the ryanodine receptor/Ca^{2+} release channel[1]. It contains a single transmembrane domain that separates the protein into a cytoplasmic and a luminal segment[2]. Wei and Campbell[3] suggest that triadin mediates the functional coupling between the ryanodine receptor/Ca^{2+} release channel and calsequestrin in the lumen of the sarcoplasmic reticulum. We have isolated a phage, designated λ5, from a porcine genomic DNA library cloned in lamba FIXII (Stratagene, Heidelberg, Germany) coding for triadin. The partial genomic structure of the triadin gene was determined by sequencing of phage λ5. Within the last intron of the triadin gene a $((CT)_{21}*(CA)_{14})$ dinucleotide repeat was identified. The DNA sequence of the triadin gene was submitted to the EMBL data library under accession number AJ224992.

Table 1. Polymorphic information content, heterozgosity, allele sizes and frequencies of the triadin gene microsatellite

Repeat sequence	$((CT)_{21}*(CA)_{14})$
PIC	0·88
Heterozygosity	0·89
Allele size (bp) and frequency	234 (0·01)
	232 (0·07)
	230 (0·05)
	228 (0·16)
	226 (0·09)
	224 (0·17)
	222 (0·07)
	220 (0·03)
	218 (0·01)
	214 (0·01)
	210 (0·02)
	208 (0·15)
	206 (0·08)
	204 (0·02)
	202 (0·03)
	190 (0·02)
	186 (0·01)

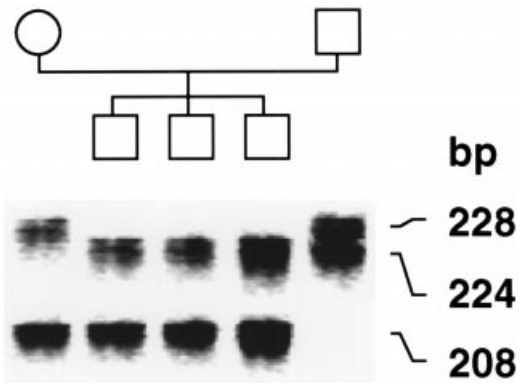

Fig. 1. Fluorogramy showing Mendelian inheritance in a family of Angler Saddlebacks. Allele sizes are indicated.

Primer sequences:

Forward primer (TRIMS1 f): 5′-GCT TAC AGG AAT CTA CTG G-3′

Reverse primer (TRIMS2 r): 5′-GAC TTT AGG AGA CAC AGG-3′

PCR conditions and analysis: PCR reactions were optimised using the 'Ready to Go' system (Pharmacia, Freiburg, Germany). A master mix was prepared containing 400 μM of each primer (the forward primer was labelled with IRD 800 fluorescence dye; MWG Biotech, Ebersberg, Germany), frozen and stored at –20 °C. DNA (100 ng) in 1 μl TE (10:1) was mixed with 12 μl master mix and one PCR bead was added (10 mM Tris–HCl pH 9·0, 50 mM KCl, 1·5 mM $MgCl_2$, 200 μM of each dNTP and 1·5 U *Taq* polymerase). Cycling included an initial denaturation step at 94 °C for 10 min, 30 cycles of 1 min at 94 °C, 30 s at 55 °C, 72 °C for 1 min and a final step of 10 min at 72 °C using a Hybaid Omnigene cycler. PCR products were analysed by electrophoresis on 8% denaturing polyacrylamide gels using a LI-COR Gene ReadIR 4200 automated sequencer. Analysis of the bands was performed using the RFLPscan Plus 3·0 (Scanalytics, Virginia, VA) software.

Polymorphism and allele size: Polymorphism was studied using DNA samples of 77 Angler Saddleback, five German Large White, five German Landrace, five Pietrain, and six Bunte Bentheimer pigs. Among these 98 pigs 17 different alleles of the microsatellite were detected. The lengths of the corresponding PCR products were 234, 232, 230, 228, 226, 224, 222, 220, 218, 214, 210, 208, 206, 204, 202, 190, and 186 bp. Heterozygosity, PIC values, and allele frequencies are shown in Table 1.

Mendelian inheritance: Co-dominant segregation of the polymorphisms was observed in 48 individuals from five families of Angler Saddlebacks (Fig. 1).

Chromosomal location: The porcine triadin gene was assigned to chromosome 1 by PCR screening of a porcine rodent somatic cell hybrid panel[4].

Acknowledgements: The authors are grateful to A. Deppe for expert technical assistance.

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Nine canine microsatellites

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Source/description: Partial genomic libraries were constructed in *Sma*I-cleaved M13mp10 using *Hae*III-cut DNA from several dogs and screened with a poly (dA-dC).poly(dG-dT) probe (Pharmacia, Uppsala, Sweden) labelled using deoxyadenosine 5′-[α-^{32}P]triphosphate (Amersham, Buckinghamshire, UK). Positive clones were sequenced using a Sequenase kit (Amersham, Buckinghamshire, UK). Primers flanking the dinucleotide (CA/GT) repeats were selected using the program PRIMERS (see Table 1).

PCR conditions: PCR conditions are shown in Table 1 with PCR carried out as described previously[2].

Heterozygosity: Heterozygosity was calculated using the program PIC (see Table 1). A minimum of nine unrelated dogs of mixed breeds were typed for each microsatellite.

Allele sizes: Allele lengths were estimated by comparison with an M13 sequence ladder on 5% denaturing acrylamide-urea gels. The product amplified from the appropriate M13 clone was run at the same time.

Chromosomal location: Unknown.

Acknowledgements: We are grateful to Dr K.C. Barnett, Mrs P.J. Rothoff-Rook and Ms D.J. Flack for help in obtaining blood samples. Work on the canine genome at the Animal Health Trust and at the University of Leicester is supported by the Guide Dogs for the Blind Association. PRIMERS and PIC were made available through the Human Genome Mapping Project Resource Centre (Wellcome Genome Campus, Hinxton, Cambridge, UK).

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Table 1. Characterization of nine canine microsatellites

Microsatellite number	Primers	Clone	H[a]	Number of alleles	Conditions[b]	PCR product length (bp)
AHT116	5′ATTCAGTCAATATGCAGGCT3′ 5′CTATGCTGTGAGGCTTTCCA3′	$(GT)_{16}$	0·11	2	2 mM/54	229–231
AHT128	5′AGTTTGAAGCAGCATGGTAGTG3′ 5′CGCAGTCGCATGTGTGTCTA3′	$(GT)_{18}$	0·76	6	1 mM/TD59	80–90
AHT140	5′GTCGTGCATGGGAAAATTCT3′ 5′TATGGTCCAGGCTGTGAACA3′	$(GT)_{17}$	0·81	8	2 mM/TD59	101–117
AHT142	5′GGTGAGGCACTTAGCTTTTCC3′ 5′AGAAATTACCCAAACCTAGCCC3′	$(TG)_{14}$	0·69	5	2 mM/TD59	188–198
AHTf12	5′CAAGCTTTCTGAAAATGGTGC3′ 5′CTCTGCCTGTCTCTGCCC3′	$(CA)_{15}$	0·77	5	2 mM/TD62	132–146
AHTf16	5′GAGGTGGGGGTATAGGAAGC3′ 5′CCTGCTTCTCCCTCTGCC3′	$(CA)_{24}$	0·79	6	2 mM/TD59	155–165
AHTf44	5′CCCGAGATACAGGAAATGGA3′ 5′CCTGCTGCATGGTGTTACAC3′	$(GT)_{17}$	0·74	4	2 mM/TD59	153–159
AHTf64	5′CACTCCCCAGGACTGCAG3′ 5′GGGTTACTGAGCAGAGCCTG3′	$(TG)_{16}$	0·71	4	1 mM/TD61	118–124
AHTf66	5′CAAAGGCTTCCCATCTGTC3′ 5′TCGGTTACTCATATCTGGGG3′	$(CA)_{24}$	0·74	5	1 mM/TD58	171–179

[a]Heterozygosity (unbiased)[3].
[b]TD are Touchdown programs[1].