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Radiation hybrid mapping of the pig *ALDOA*, *ALDOB* and *ALDOC* genes to SSC3, SSC1 and SSC12

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Source/description: Fructose-1, 6-bisphosphate aldolase is a glycolytic enzyme which plays a critical role in the reversible conversion of fructose-1, 6-bisphosphate to glyceraldehydes 3-phosphate and dihydroxyacetone phosphate. It is reported that in vertebrates fructose-1, 6-bisphosphate aldolase has three isozymes: aldolase A, fructose-bisphosphate (ALDOA); aldolase B, fructose-bisphosphate (ALDOB) and aldolase C, fructose-bisphosphate (ALDOC). In human beings, deficiency of aldolase A has been described as a rare cause of hereditary non-spherocyte haemolytic anaemia (HNSHA); deficiency of aldolase B was involved in hereditary fructose intolerance (HFI), while the potential biological function of aldolase C has not been reported so far. In pig, *ALDOB* has been previously localized on chromosome 1q18 or 1q28–q213 using somatic cell hybrids,[1,2] and this location has been narrowed to 1q28–q29 by the fluorescence *in situ* hybridization.[3] The objective of the present study was to map *ALDOA*, *ALDOB* and *ALDOC* on the porcine radiation hybrid map.

Primer design, PCR condition and sequencing: The human mRNA sequences (see Table 2) were employed to screen the porcine expressed sequence tags (ESTs) in EST-others database through standard BLAST (http://www.ncbi.nlm.nih.gov/blast/) algorithm. The porcine ESTs sharing at least 80% sequence identity with the corresponding human mRNA were used for primer design. The amplified products were obtained in 20-μl reaction volume consisting of 50 ng of porcine genomic DNA, 1× PCR buffer, 0.3 μM of each primer, 75 μM of each dNTPs, 1.5 mM $MgCl_2$ and 1 U *Taq* DNA polymerase (Promega, Madison, WI, USA). The PCR parameters were: 5 min at 95 °C followed by 40 s at 94 °C, 30 s at 58–61 °C and 30 s at 72 °C for 35 cycles and a final extension of 7 min at 72 °C. The PCR products were purified with Wizard prep PCR purification system (Promega), cloned to Promega pGEM-T easy vector and sequenced by a commercial service. Comparisons of pig predicted exonic sequences with the human corresponding mRNA sequences in the GenBank were done using the BLAST2 software provided by the NCBI server (http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html) to assure that the pig-expected sequences were isolated. The primer sequences, GenBank accession numbers (pig), similarity to human sequences, primer binding region, annealing temperature and PCR product size for the three genes are presented in Table 1.

Chromosomal location: The porcine radiation hybrid panel, IMpRH,[4] was used for regional mapping using the pig-specific primer pairs (PF2–PR2, PF3–PR3 and PF4–PR4 in Table 1). The PCR reactions were performed in 10 μl, on 25 ng of DNA from each hybrid line (118 clones) and on 25 ng of genomic DNA from pig and hamster. A negative control containing no DNA template was also performed. The PCR products were scored on a 2.0 % agarose gel stained with 0.5 μg/ml ethidium bromides. The PCR results for the three genes were analysed using the IMpRH mapping tool[5] (http://imprh.toulouse.inra.fr/). The three genes were significantly linked (LOD > 6) to a marker of the first generation radiation hybrid map.[6] Their chromosomal locations (Table 2) were inferred based on the positions of the closely linked markers directly localized on the cytogenetic map; if not, estimated by the positions of the proximal markers previously assigned to the cytogenetic map. The markers that have been used to propose a cytogenetic position are indicated in Table 2.

Comments: In human beings, *ALDOA* has been located to chromosome 16q22–q24 (http://www.ncbi.nlm.nih.gov/Locuslink/), while the human genome sequence draft reports its position on the short arm of this chromosome (HSA16p11.2) (http://bioinfo.weizmann.ac.il/cards/index.html). In our study, we assigned pig *ALDOA* to SSC3p14, which was consistent with comparative mapping results[7] and in favour of a position on the short arm of HSA 16. We found that the marker *SSC1F12*

Table 1 The primers used for isolating and mapping the three genes.

Gene symbols	GenBank acc. no. (porcine)	Sequence similarity[2]	Primer sequences (5′-3′)	Primer binding region	Size (bp)	Tm value (°C)
ALDOA[1]	AY359812	93%	PF1-GAACCAACGGCGAGACAA	Exon5	898	60
			PR1-GGTGCCTTCCAAGTAGATGTG	Exon8		
			PF2-CTCCTCCTGCTTCGGTGTC	Intron6	205	60
			PR2-GACAGACTCCTGGCTTCACG	Intron6		
ALDOB	AY359813	92%	PF3-ATTTGGAGGGCACTCTGTTG	Exon7	616	58
			PR3-AGGTTGATAGCATTGAGGTTGAG	Exon8		
ALDOC	AY359811	93%	PF4-CACGGAGGAGTTCATCAAGC	Exon8	376	61
			PR4-TGACTCAGTGCTGGATGGAG	3′ UTR		

[1]The primer pair PF1–PR1 was used to isolate the 898-bp fragment of *ALDOA* gene genomic DNA. The pig-specific primer pair PF2–PR2 was used for physical mapping, which was selected from the intron region of 898 bp porcine fragment above.

[2]The predicted exonic sequences of the pig were compared with the corresponding human sequences.

Table 2 The chromosomal assignments of the three porcine genes.

Gene symbol	Human acc. no.	Human cytogenetic location[1]	Porcine RH mapping result				
			Retention (%)	Closest markers	Distance (cR)	LOD score	Porcine cytogenetic position[2]
ALDOA	NM_000034	16q22–q24	40	*SW1443*	65	6.12	3p14(*S0206*)
ALDOB	NM_000035	9q21.3–q22.2	26	*SSC1F12*	17	17.58	1q28–1q29 (*SSC1F12*)
ALDOC	NM_005165	17cen–q12	15	*SWC23*	30	10.8	12q13(*S0106*)

[1]The human cytogenetic locations come from the website: (http://www.ncbi.nlm.nih.gov/Louslink).

[2]The closest or proximal marker that has been used to deduce a cytogenetic position are present in brackets.

(porcine EST with the Genbank accession number: F14528) was significantly linked (two-point analysis) to *ALDOB*. This marker that shares 90% sequence identity with the human *ALDOB*, has been mapped by others to SSC1q28–1q29.[1–3] We have previously mapped ferredoxin reductase (*FDXR*) and rod cGMP-phosphodiesterase γ-subunit (*PDE6G*) genes to SSC12p,[8,9] while we report here the assignment of porcine *ALDOC* gene to SSC12q. Altogether, these chromosomal assignments confirm the established conservation of synteny between the HSA17 and SSC12.[7]

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Assignment of the chicken *glycoprotein hormones, alpha polypeptide* (*CGA*) gene to chromosome 3

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Source/description: The pituitary glycoprotein hormones (luteinizing hormone, follicle-stimulating hormone and thyroid-stimulating hormone) are composed of an alpha subunit and a beta subunit. The chicken glycoprotein pituitary hormone alpha subunit (CGA) is identical in all three hormones, but the beta subunit is hormone specific. These hormones are known to exert considerable control on growth, metabolism and reproduction. DNA from Red Jungle Fowl (RJ; UCD-001) and White Leghorn (WL; UCD-003), parents of the East Lansing reference mapping population (EL),[1] was used for mapping the *CGA* gene.

Primer sequences: Primers were designed from the published cDNA sequence.[2]
Primer 1: 5′-ATGGATTGCTACAGGAAGTAT-3′
Primer 2: 5′-TGCTACACAGCACGTTGCTTC-3′

PCR conditions: Each 20 μl reaction contained 1X buffer, 1.5 mM $MgCl_2$, 100 μM dNTPs, 0.2 μM for each primer, 0.5 units of Bioline Taq Polymerase and 100 ng of DNA. A Peltier Thermal Cycler, PTC-225 (M J Research Inc., Waltham, MA, USA) was used with the following settings: 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 59 °C for 1 min and 72 °C for 2 min, and then terminated with 72 °C for 7 min.

RFLP analysis: A 1753-bp fragment was amplified from the RJ and WL DNA. The fragment was gel purified using a Qiagen MinElute Gel Extraction Kit (Qiagen Inc., Valencia, CA, USA) and sequenced on an ABI 3100 Genetic Analyzer (Applied Biosystems Group, Forester City, CA, USA). The DNAStar Inc. program (Madison, WI, USA) was used to compare the RJ and WL sequences (GenBank accession number AY436327). A restriction digest of the amplified fragment using *Nla*III was performed at 37 °C for 1 h, and the fragments separated by agarose gel electrophoresis (1%). Three polymorphic *Nla*III sites were identified (Fig. 1). The following bands were detected in

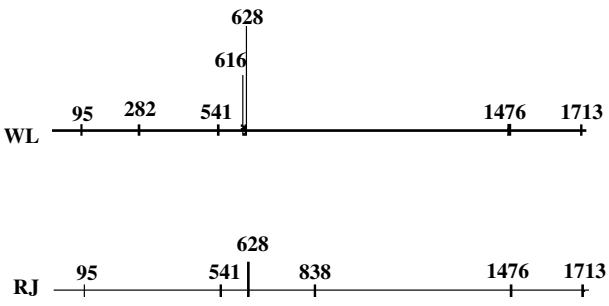

Figure 1 *Nla*III restriction sites in a 1753-bp fragment of *CGA* in White Leghorn (WL) and Red Jungle Fowl (RJ).

RJ: 87 bp, 95 bp, 210 bp, 237 bp, 446 bp and 638 bp. The following bands were detected in WL: 95 bp, 187 bp, 237 bp, 259 bp and 848 bp.

Mapping: The Map Manager[3] program was used to establish linkage between *CGA* and other loci on chromosome 3 in the EL population (LOD = 12.0). It was located 7.7 cM distal to *ADLO115*, and 3.9 cM proximal to *malic enzyme 1* (*ME1*) gene.

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Radiation hybrid mapping of 75 previously unreported equine microsatellite loci

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Source/description: Horse genomic DNA was digested with the restriction enzyme *Mbo*I, size selected by gel electrophoresis for fragments between 200 and 1200 bp, and ligated into the *Bam*HI site of the M13 phage vector. Clones containing a potential microsatellite were identified by screening the library with [^{32}P] 5′ end-labelled oligo $[dCA]_{16}$ and oligo $[dGT]_{16}$ probes. The DNA was isolated from positive plaques and the inserts were sequenced using an ABI 3100 automated sequencer. Primer pairs for PCR amplification of the markers were developed using the PRIMER program (Version 0.5; M. J. Daly, S. E. Lincoln and E. S. Lander, unpublished). Sequence accession numbers, repeat motif, PCR primer pairs and product sizes based on the cloned sequences are provided in Table 1.

PCR conditions: Polymerase chain reactions for determination of microsatellite polymorphisms were performed in 15 μl volumes consisting of the following reagents: 25 ng DNA; 1X PCR buffer (Qiagen, Valencia, CA, USA); 1.5 mM $MgCl_2$; 25 μM each of dCTP, dGTP and dTTP; 6.25 μM dATP; 0.125 μCi [α-^{32}P] dATP; 0.45 U HotStar *Taq* polymerase (Qiagen) and 5 pmol of each primer. Amplification was done using a MJ Research PTC100 thermocycler (Watertown, MA, USA) under the following conditions: initial 20 min denaturation at 95 °C; 30 cycles of 94 °C for 30 s, annealing temperatures that ranged from 54–62 °C for 30 s and 72 °C for 30 s; and a final 5 min extension at 72 °C. Reaction products were electrophoresed through 7% acrylamide denaturing gels on BioRad SequiGen GT 38 cm ×50-cm plate sequencing gel units (Hercules, CA, USA), in the presence of 1X TBE, and fragments detected using autoradiography.

The PCR for typing markers on the 5000 rad whole genome equine radiation hybrid panel comprising 92 horse × hamster hybrid cell lines[1] contained 50 ng DNA; 5 pmol of each primer; 2.0 mM $MgCl_2$; 0.3 U HotStar *Taq* Polymerase (Qiagen) and 10X PCR buffer which contained Tris-Cl, KCl and $(NH_4)_2SO_4$ (Qiagen). Markers were typed in duplicate, separated by electrophoresis on 2.5% agarose gels, and scored manually.

Allele frequencies: The DNA from 12 stallions of the Equine Genome Mapping Workshop International Reference Family,[2] and/or three horses (one stallion and two mares) from the parental generation of the Newmarket full-sibling reference family,[3] was amplified. The number of alleles observed in this sample population ranged from 1 to 10 (Table 1).

Radiation hybrid mapping and chromosomal assignments: The PCR typing of the 75 microsatellite markers on the 5000 rad equine RH panel and data analysis was performed as described in detail by us.[1] Two point analysis with the RHMAPPER software was carried out to assign markers to map positions on the current equine radiation hybrid map[1] at LOD ≥ 12.0.[1] Chromosomal assignments and the nearest linked marker for all new equine microsatellites are provided in Table 1.

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Table 1 Equine microsatellite loci *UMNe84–UMNe376*.

Marker	Accession number	Repeat motif	Primer 1 (5′ → 3′)	Primer 2 (5′ → 3′)	Product size (bp)	Heterozygotes	Alleles	Chromosome (by RH mapper)	Closest Marker(s) (by RH mapper)	Distance from closest marker(s) (cR)
UMNe84	AY391284	CA8 N2 CA5	ACAGTTGGATATCCGTGGGAAC	TCTGTTATGTGTTGCCCTCTGC	216	0	1	ECA15	*COR075*	0.00
UMNe90	AY391285	CA18	CGACACTTCCCACAGTCATCTC	TCCGTGCTTTTAAGCAAGTTCC	149	0	1	ECA1	*UCD487*	17.67
UMNe91	AY391286	CA19	GCAACAATAAGATACCCAAAGCAG	GATCTTGGCCAACAACTCGTC	153	7	5	ECAX	*ARSC1*	11.32
UMNe93	AY391287	CA13 N2 CA8	CGCAGAGTTGGAGACACCTG	GGTGCCATCCACTGAAACAC	167	2*	3	ECA7	*LEX045*	0.00
UMNe97	AY391288	CA5 N2 CA5	TGTCAGGTGGCATTCAAATC	TAACCAACAATGCCCAAGTC	202	0	2	ECA18	*NV077*	0.00
UMNe100	AY391289	CA5 N8 CA6	CTGATGCAGAGGCATTTCTG	CGTCAGCTGAGGCAACTATG	183	5	5	ECA7	*HBB*	2.84
UMNe102	AY391290	CA9 N4 CA9	ATGCATGCTCATTGCAGAAC	GCACAAAGAAATTTCCAACTCC	191	0	1	ECA15	*LEX046*	0.00
UMNe104	AY391291	CA15	TGATTTTTGTTCAGCCTCTATGC	CCTAGAAAGGTGGGAAATAAATTG	156	0	1	ECA4	*HTG07*	0.00
UMNe106	AY391292	CA7 N CA3	AATCCTGTGAGGCAGGAGTG	TGTCTGGGCATCTCTTAGGG	200	0	1	ECA19	*AHT052*	11.88
UMNe110	AY391293	CA11	TCTTTCCTACCCTCCATGACC	GATCTAGTACAACACTCCGTGTG	141	9	5	ECA4	*PTPN12*	0.00
UMNe112	AY391294	CA9	TGCCCAAATATTGAGCAAGG	TCTCTCCTCCCCTTTTTGTG	155	0	1	ECA4	*LEX033*	5.23
UMNe114	AY391295	CA5 N2 CA7	AACCCAAAAAGCATTTGAGAAG	TCTGCACCAGTTTTGTACGC	129	1	2	ECA18	*SG07*	19.85
UMNe115	AY391296	CA12 N2 CA27	TCCTCCTACACTGGCCATATC	TTTCCTATCGGAGTGCTTGC	168	4	10	ECA1	*MYO5A*	11.09
UMNe125	AY391297	CA14	TGGGTCCTGAGACCATAAGC	TCCTCCCTACCTCCTCACTG	143	9	5	ECA14	*LEX078*	0.00
UMNe127	AY391298	CA10	TTATAAATCACCACTGTTTACACAC	TCTTGAAGCAGGATGGGC	135	0	2	ECA26	*TKY275*	30.11
UMNe137	AY391299	CA10	CTGCTTTTTACTGCTTCAGTGC	GATTTGAGTCGAGGTCTGCC	114	5	5	ECA22	*HMS47*	2.22
UMNe139	AY391300	CA13	AGACACAGGTTTAGGTGGATGC	GATCAAGCACATAAGGGACAC	100	5	2	ECA21	*C9*	2.53
UMNe143	AY391301	CA13	AGAAAGGGTAAACAAGAAGCCC	GACACCTCTGCATCACACATG	105	3	2	ECA11	*NV090*	2.74
UMNe147	AY391302	CA10	CAGACCTACTCCAGTCATCAGC	AAACAAAGAGACTTGAAGTGGC	180	0	1	ECA21	*HTG10*	8.23
UMNe152	AY391303	CA11	ACAGGTGTTCATCCAAATTTTG	AAAGTAGAGGAAGACAGGCACG	144	5	2	ECA24	*COR087*	0.00
UMNe157	AY391304	CA11	TTCAACTGTGTGAGTGTGAATG	AGCTTTTGTCCAGCAGGAAC	94	5	3	ECA31	*PCMT1*	5.23
UMNe158	AY391305	CA22	AATTGAGAGCCAAGATGACACC	GGCACCATTTGAGGAAGATG	146	8	8	ECA3	*KCC1*	4.60
UMNe160	AY391306	CA7 A2 CA15	TGGAAGGATTCTCCCCAAG	GTTGCTCCAATGCCATACTC	114	0	1	ECA25	*NV043*	9.10
UMNe164	AY391307	CA9	AAAGAGGAGGATTGGCAATG	ATCTCCAGTGTGACAATCTCTACC	105	0	1	ECA8	*DSC2*	11.43
UMNe168	AY391308	CA10	CACCAAACCCCACTGAATTC	CACTACCTTCCCCTACGTTCC	149	1	2	ECA12	*IGF2*	20.58
UMNe170	AY391309	CA7 N2 CA5	GGGTGTTAAGAATCCTGCTCC	CTAGGCAAACTACTGACCCCC	138	0	1	ECA20	*UM011*	0.00
UMNe172	AY391310	CA4 N2 CA6	CCAGACACAGACCTAGCACC	CCAGCAAAGGAGGAGGAAG	190	0	1	ECA4	*HMS62*	6.72
UMNe173	AY391311	CA11	TTGGAGAATTCAAGGGTTTCC	GCCAAGACATGGAAACAACC	132	7	5	ECA17	*COR032*	13.35
UMNe178	AY391312	CA11	TGGCAGATAAAGCCCTTACC	GATCACATAGTGAAGGTGTTAAACG	107	1	2	ECA3	*LEX007*	0.00
UMNe180	AY391313	CA8 N2 CA2	TGGAAAATCCTCACAAACTGC	TATATTTTCCTTTTGCGTGTGC	148	0	1	ECA18	*CHRNA*	5.34
UMNe181	AY391314	CA6 N CA5	TTAAGCAACCCAGTGTGTGG	AGAGAAGAGAATCGGGGGAG	149	0	1	ECA31	*PCMT1*	7.80
UMNe182	AY391315	CA10	TCTCTGCATTTCACTTCTCGG	TGAGAGAAGAGCCCAGGAAG	132	3*	3	ECA3	*KCC1*	4.60
UMNe183	AY391316	CA11	AGAGAACAGGAGAAGACATGCC	ACCCTGTCTCATGGAAGCTG	106	1	2	ECA22	*ADA*	11.32
UMNe188	AY391317	CA8 N CA2	GTTAACAAGGATTGTTTTGGGC	TGCGTTTCTGCTTCTCCC	132	2	2	ECA26	*EB2E8*	18.15
UMNe195	AY391318	CA12	CAAAGAAGTGGCCCTCGTAG	GATCTGGCCCCAGAGAAAAA	104			ECA6	*MAP2*	11.32
UMNe196	AY391319	CA16	ATGAAAAAAGGCATACCCCC	TCTTATCAGGTTGATGGTGTGC	131	7	5	ECA1	*RET*	14.73

Table 1 Continued.

Marker	Accession number	Repeat motif	Primer 1 (5′ → 3′)	Primer 2 (5′ → 3′)	Product size (bp)	Heterozygotes	Alleles	Chromosome (by RH mapper)	Closest Marker(s) (by RH mapper)	Distance from closest marker(s) (cR)
UMNe217	AY391320	CA11	CTTTGAGTTCACCAGTTCTCCC	AACCAAAAGGAACTTTGGTGG	151	3	2	ECA13	*TKY031*	8.34
UMNe223	AY391321	CA10	GTCTGTCCCAGACATATACCCC	AACCTGGGCAAGGGAAAG	112	0*	1	ECA12	*TKY286*	0.00
UMNe225	AY391322	CA17	GATCTCTTGCTGTGTGTTTGTG	TCAATTTCACAAGATGGAAACG	154	2*	4	ECA20	*UM011*	0.00
UMNe227	AY391323	CA13	ATAATTTCCCTTGCCAACACC	CTGTAGACCCAAAGGAAGATGG	188	1*	2	ECA14	*COR104*	41.70
UMNe230	AY391324	CA11	TGGTCTAGAGTACACATACACCAAC	TTTCTCCTTCCACCCCAG	109	0*	1	ECA5	*AHT024*	0.00
UMNe235	AY391325	CA17	TGAAGGCAATGACCAATATCC	CACCAACAAAAGTATCTGCCTG	178	2*	2	ECA17	*LEX055*	13.47
UMNe238	AY391326	CA11	CCAGCACCACTGTTCAAGAC	TCTAGCCATATGTTACCCTGCC	198	0*	1	ECA8	*ASB14*	12.33
UMNe245	AY391327	CA10	TTGCATAGGGACAATCCTACG	ACAAACCCTACCACTCGGC	104			ECA15	*ASB19*	0.00
UMNe253	AY391328	CA23	CAGAAAGATTTCCTTCTTTATGGC	ACTACACTGCCTCTTGGAATATCC	222			ECA29	*LOC91452*	1.41
UMNe256	AY391329	CA14	GGCCACACGACTACTTTCAC	ACTTCTGGAAGACAGCTGGC	142	2*	3	ECA4	*COR047*	10.09
UMNe264	AY391330	CA12	GCAATGCTAGCACAGGGG	CCTCTCTCTCTTTCCCCCTC	101	8	3	ECA16	*PDCD6IP*	7.26
UMNe276	AY391331	CA17	CATGCTCTCCATGACTTTTCC	CACCCAGGACTCCCACAG	154	2*	3	ECA22	*AHT030*	11.65
UMNe301	AY391332	CA5 N2 CA5	TTTTGGGGTCCAGAATAAACC	TAGACAACCAAATGCCAAAGG	140	4	3	ECA20	*NV005*	2.22
UMNe305	AY391333	CA13	ACAGTTCACAGCGGCCTC	GAGAGGGAGCAAGCCCTC	162	2	2	ECA13	*TKY031*	7.04
UMNe313	AY391334	CA15	AGAACCTGTTGGAGATACGAGG	ATCAGAGTGGAGACATGGGG	179	7	5	ECA27	*COR040*	14.62
UMNe314	AY391335	CA10	AGGCCCCTTGTATATCCCAC	GAGCTGGGAGTGGAAGGAG	153	0	1	ECA11	*UCD039*	0.00
UMNe318	AY391336	CA4 N2 CA7	CAGGGCTAGTCAACAGGCTC	TGGGGTGTAATGACTACCAGG	209	0	1	ECA1	*COR046*	4.71
UMNe319	AY391337	CA10	TTCGTGCAATCTGTTGCTTC	GATCACAGTCCCTGGTACTGG	154	1	2	ECA19	*HTG24*	0.00
UMNe323	AY391338	CA8 CA8 CA4 CA6 CA14	GATCCTGCAGGAAAGCATGT	CCGCTCGGAATATTTCATTG	185	4	7	ECA2	*COR037*	0.00
UMNe327	AY391339	CA16	TTTTCCTTCCTCATTGGTGC	GAAATGCAGGGCTAAGGATG	157	4	2	ECA21	*C9*	2.33
UMNe329	AY391340	CA19 N2 CA8	TGTTTGCTTGGAATGGTCAG	TCTCCATCTCCACATAGGCC	104	4	3	ECA12	*TKY286*	8.12
UMNe330	AY391341	CA10	AAACATGGAACCAGAGGGG	TCTCCAGCGTATTTGGTTAGTG	129	0	2	ECA2	*FABP3*	5.55
UMNe337	AY391342	CA17	CTTTAATATGCCTGCCACTTCC	TTGGGAAGTCCAGGATCTTG	215	2	3	ECA6	*CUL3*	0.00
UMNe350	AY391343	CA10	GCAAAATAAAAGGGTCACTTGC	AGTGCTCCAGGTGCTTATATCC	153	6	2	ECAX	*COR091*	0.00
UMNe351	AY391344	CA14	TGAGAAGCTCTTGCCAATCC	TAGAGGCCATTTTTGCATCC	150	0	1	ECA19	*HTG23*	2.43
UMNe353	AY391345	CA19	CCACTTTTCCCCTTCCTCTC	AACTTGGAAGCAACCTAAGTGC	322	2*	3	ECA6	*UCD465*	0.00
UMNe355	AY391346	CA13	TCATGCAATTCTGAAAAAGAGC	CAGAGCAAATCTTCCTCACC	182	2*	2	ECA22	*TKY285*	12.90
UMNe357	AY391347	CA10	GATCCAGTAACAAACAAGCTAATTT	TCAAATGATTTCTTACTGAGGATTC	210	1	2	ECA23	*UMNe51*	0.00
UMNe359	AY391348	CA10 N2 CA5 N2 CA20	GTGTGACGGAGGACGAGG	TGGTGCCATCCACTGAAAC	140			ECA7	*LEX045*	0.00
UMNe361	AY391349	CA14	AGGGAATAACTTATGAACAGATGAA	TCTTTATGGAAATTCTCTCTGAACC	169	2*	3	ECA5	*HMS05*	5.13
UMNe362	AY391350	CA10	GAGAGAGAGAGTATGCGCGC	GATTCCAAAGGCTATCCACTC	117	2*	3	ECA25	*TXN*	19.48
UMNe365	AY391351	CA10	CCCTCGTCTCAGACTTCTGG	GAGGCCTTGCATGTGGAC	201	0*	1	ECA11	*PDE6G*	0.00
UMNe366	AY391352	CA11	TGAACTCCAAAGCCAGGC	CATGTTCTGTAGTTTTGCAGGC	174	0*	2	ECA29	*TKY112*	7.15
UMNe368	AY391353	CA15	TCCCAGAAAGCAACACTCAC	TGTCAGTTCAGATTTTTGGCC	292	0*	1	ECA31	*VIP*	0.00
UMNe372	AY391354	CA11	ACGTTACAGGTGGGGAGATG	CTTGGCTTCCAGGAAAACAC	127			ECA10	*CASP8AP2*	3.67
UMNe373	AY391355	CA17	AGGCCACTCAAGCCACAG	CACCTCCTCCTCCAAATGAG	265	9	4	ECA15	*ASB19*	0.00

UMNe374	AY391356	CA16	AATTTCATATGGTTTCCATGCC	TAAGCACTGCGTTAATGTTCTG	194	2*	3	ECA2	*SMARCA5*	31.61
UMNe375	AY391357	CA6 N2 CA12	GGAGCCAGTTCAAAGATTTCC	ATTCAGTGGGTTGTTTTGCC	130	2*	2	ECAX	*FACL4*	3.98
UMNe376	AY391358	CA21	ACGCATATTAAAGTTTGAGAAGTCC	CAGACCTACTGAATTGGAATACACC	155	2*	5	ECAX	*COL4A5*	3.15

Repeat motif, PCR primers and product sizes, polymorphism and radiation hybrid map location (chromosomal assignment along with closest located marker and distance in cR) for these new microsatellite markers were obtained as described in the accompanying text. An asterisk (*) by the number of heterozygous individuals indicates that these markers were only typed on the Animal Health Trust reference family parents.

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Assignment of the *phosphoglycerate kinase 2* (*PGK2*) gene to porcine chromosome *7q14–q15* by fluorescence *in situ* hybridization and by analysis of somatic cell and radiation hybrid panels

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Source/description: Phosphoglycerate kinase (PGK) (ATP: 3-phospho-D-glycerate 1-phosphotransferase, EC: 2.7.2.3) catalyzes the reversible conversion of 1,3-diphosphoglycerate to 3-phosphoglycerate, generating one molecule of ATP. Two functional *PGK* loci have been identified in mammalian genomes. The *PGK1* is X-linked and ubiquitously expressed in all somatic tissues. The *PGK2* is autosomal, intronless and solely expressed in spermatogenic cells.[1] The *PGK2* gene is one of the non-intron interrupted but functional genes that possess all characteristics of a processed gene including a complete open reading frame, the remnants of a poly (A) tail and bounding direct repeats.[2] As the human *PGK2* gene, linked to the HLA,[3] has been assigned to *HSA6p21.1–6p12*, mapping results of the porcine *PGK2* gene provide further knowledge about the homology between the p-arm of human chromosome *6* and the q-arm of pig chromosome 7.

Isolation of the porcine PGK2 gene from a PAC library: To generate a probe for screening of the porcine genomic PAC library TAIGP714,[4] polymerase chain reaction (PCR) amplification was performed with primers A (5′-GCC AGA TAA AAT AGA AGC C-3′) and B (5′-CAC CAC CAA TAA TCA TCT C-3′) designed from the human *PGK2* mRNA (EMBL/GenBank accession no: BC038843). The PCR was conducted using 50 ng of porcine DNA in a final reaction volume of 25 µl. The standard PCR profile was as follows: denaturation at 94 °C for 4 min, followed by 35 cycles of 94 °C for 30 s, 52 °C for 30 s, and extension at 72 °C for 30 s. The final cycle was at 94 °C for 30 s, 52 °C for 30 s, and an extension at 72 °C for 10 min. The resulting fragment of 287 bp (EMBL/GenBank accession no: AY436817) was bidirectionally sequenced using the pGEM®-T vector system (Promega, Heidelberg, Germany) and a similarity of 91% between the probe and the human *PGK2* mRNA confirmed the sequence identity. Using primers A and B the PAC clone TAIGP714A03214Q of approximately 95 kb length, harboring the porcine intronless *PGK2*, was isolated from the PAC library.

Fluorescence in situ hybridization: Metaphase spreads were prepared from peripheral lymphocytes obtained from a normal, healthy boar as described previously.[5] Probes (1 µg TAIGP714A03214Q DNA) were labelled with digoxigenin-11-dUTP by nick-translation using the DIG-Nick Translation

Mix (Roche, Penzberg, Germany). Labelled probes were hybridized with 30× excess of porcine Cot-DNA and 6 μg of salmon sperm DNA. Immunodetection was performed using Digoxigenin-antibodies conjugated to Cy3. Chromosomes were counterstained with DAPI and examined with a Zeiss Axioplan 2 microscope (Carl Zeiss, Jena, Germany). The G-like banding pattern generated by DAPI staining was used for chromosome identification and for regional assignment of the hybridization signals. Fluorescence *in situ* hybridization (FISH) experiments were carried out twice using duplicate slides.

Hybrid panel analyses: A porcine rodent somatic cell hybrid panel[6] and the INRA/University of Minnesota porcine radiation hybrid panel (IMpRH)[7] were screened for the *PGK2* gene. Primers (5′-GTT CCC TGC AAC ATC GGG ATC-3′ and 5′-GGT GGG TTG GCA CAA GCT TGC-3′) originated from the 5′-untranslated region of the porcine *PGK2* gene. The PCR amplification of a 386 bp fragment (EMBL/GenBank accession no: AY436818) was done in a total volume of 25 μl with 25 ng of panel DNA as template. After an initial denaturation step at 94 °C for 4 min, the PCR was performed in 35 cycles with denaturation at 95 °C for 30 s, annealing at 58 °C for 40 s and elongation at 72 °C for 30 s followed by a final elongation step at 72 °C for 10 min. Obtained PCR results were evaluated using programs at http://imprh.toulouse.inra.fr (radiation hybrid panel) and http://toulouse.inra.fr/lgc/pig/hybrid.htm (somatic cell hybrid panel) at INRA.

Chromosomal location: The chromosomal localization of the porcine *PGK2* gene was done by FISH. Ten well-spread metaphases were examined, all showing a signal on *SSC7q14–q15* on both chromatides of both chromosomes (Fig. 1). Analysis of the somatic hybrid cell panel revealed a significant correlation of 1.00 between *PGK2* and *SSC7* (error risk lower than 0.1% and maximal correlation of 1.00). Within *SSC7*, chromosome regions *q12–q23* and *q26* indicated equally the highest probability of 0.4944 with a correlation of 1.00. Radiation hybrid panel analysis showed that the retention frequency of the *PGK2* gene is 23%, and the most significantly linked marker (two-point-analysis) is *SW859* on *SSC7* (4 cR distance and LOD score of 24.19). Multipoint-analysis led to linkage group *SW1701-SW859-PGK2*. The cytogenetic localization of the STS-marker *SW859* is still uncertain with *SSC7q11–q23* or *q26*.[8,9] According to the FISH and RH mapping results of the porcine *PGK2* gene to *SSC7q14–q15* with its tight linkage to *SW859* (4 cR distance and LOD score of 24.19), it is inferable from this evidence that the previous physical assignment of the marker *SW859* to *SSC7q11–q23* or *q26*, should be refined to *SSC7q14–q15*.

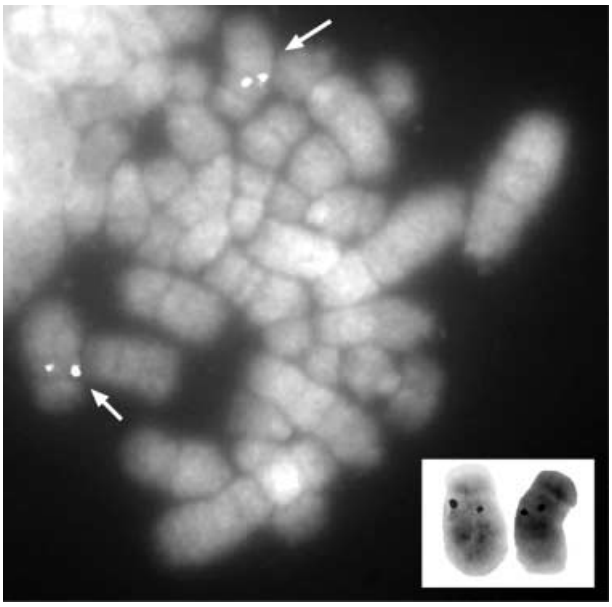

Figure 1 Chromosomal assignment of the porcine *PGK2* gene by fluorescence *in situ* hybridization analysis. Arrows indicate signals on porcine chromosome *7q14–q15*. The highlighted box shows both chromosomes in inverted colors.

Comments: The *PGK2* gene is exclusively and actively expressed in testis and the encoded protein may function as a compensatory response to the repressed expression of the X-linked intron-containing *PGK1* gene in spermatocytes before meiosis. Alternatively, it may play a beneficial role to spermatozoa.[1,2] Its physiological role makes the *PGK2* gene a functional candidate gene for inherited male fertility dysfunctions and as a putative quantitative trait locus for male fertility traits.

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Linkage mapping and expression analyses of a novel gene, *placentally expressed transcript 1* (*PLET1*) in the pig

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Source/description: To develop useful tools for functional genomics of porcine embryo development, we constructed

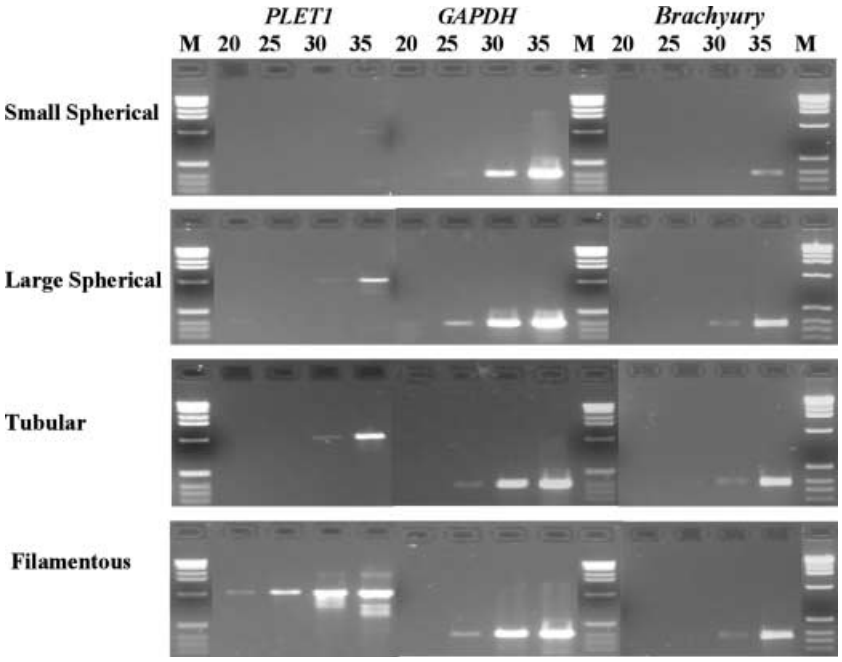

Figure 1 RT-PCR of *PLET1*, GAPDH and *Brachyury* in early gestation concepti. M: 1-kb ladder; the four lanes on the left are amplification products of *PLET1*, the four lanes in the middle are products of *GAPDH*, and the four lanes to the right are products of *Brachyury*. Embryo types were listed to the left of the picture. Lanes 20, 25, 30 and 35 correspond to 20, 25, 30 and 35 cycles of amplification from the same template.

cDNA libraries from conceptus and term placenta[1]. We identified a novel transcript PLacentally Expressed Transcript 1 (*PLET1*) expected to be highly expressed in placenta and day 14 conceptus because of the high frequency of random transcripts sequenced from the corresponding non-normalized libraries (2% of the 2107 ESTs from these tissues; see data on cluster named MI-P-E4-aii-c-03-1-UM[1]). To further characterize this gene, a putative SNP in the 3′UTR, identified from alignment of available EST sequences for *PLET1*, was confirmed by *Hinf*I digestion. This SNP was used for linkage mapping of *PLET1* in PiGMaP families[2]. Expression in preimplantation embryos of *PLET1* during early gestation was used to confirm the expression pattern predicted by sequence frequency analyses. These analyses included *GAPDH* as a housekeeping gene control and *Brachyury* (*T*) as an internal control as Brachyury has been shown to be differentially expressed during the peri-implantation period[3].

Primer sequences: For linkage mapping, a 433-bp product in the 3′UTR of porcine *PLET1* was amplified by the following primers:
Forward: 5′ GTGAATGAAGGTCTTCCGT 3′
Reverse: 5′ GCCCACATAAACGAATCAA 3′

For expression analyses, a 1066-bp cDNA was amplified from concepti RT product using the following primers from *PLET1*:
Forward primer 5′ CAAGAATCCAGGTCAACCCA 3′
Reverse primer 5′ TTGATTCGTTTA TGTGGGC 3′

GAPDH primers was used as control amplification:
Forward: 5′ CCATGGAGAAGGCTGGG 3′
Reverse: 5′ CAAAAGTTGTCAT GGATGACC 3′.

Primers and PCR conditions for *Brachyury* were as previously described[3].

PCR conditions: For linkage mapping, the PCR was carried out in a 10 μl reaction volume containing 12.5-ng porcine genomic DNA. The PCR profile was 3 min at 94 °C followed by 35 cycles of 45 s at 94 °C, 45 s at 56 °C, 45 s at 72 °C and final extension of 5 min at 72 °C. For expression analyses, total RNA was isolated from pooled small spherical (0.1–0.3 cm), large spherical (0.7–0.8 cm), tubular (2.1–4.5 cm) and filamentous (>10 cm) embryos using Trizol reagent according to the manufacturer's instructions. Two to five embryos were pooled for each RNA preparation. An additional acid–phenol extraction was conducted to remove any genomic DNA. Reverse transcriptase PCR (RT-PCR) was employed to detect *PLET1* expression in concepti by using 0.5 μg RNA from each type of conceptus. The PCR profile for both *PLET1* and *GAPDH* was 3 min at 94 °C followed by 35 cycles of 45 s at 94 °C, 45 s at 62 °C, 45 s at 72 °C and final extension of 5 min at 72 °C. A 5-μl aliquot of each reaction was removed at 20, 25, 30 and 35 cycles of PCR. The RT-PCR was repeated once for *PLET1* and *GAPDH*.

Polymorphism/Mendelian inheritance: Allelic forms of *PLET1* were identified as T (279-bp and 154-bp) and C (220-bp, 154-bp and 59-bp). Mendelian inheritance was observed in the three generation PiGMaP reference families.

Chromosomal localization: Pair-wise linkage analysis using CRIMAP2.4 was performed for all loci, with LOD scores of ≥3.0 considered significant. *PLET1* was significantly linked with 11 markers on the SSC9 linkage group. The best gene order produced by multiple linkage analysis is as follows (with distance in Kosambi centimorgans): *SW911*-(4.1)-*S0602*-(17.3)-[*CRYAB, PLET1, PPP2ARB*]-(8.3)-*APOA1*-(10.6)-*S0095*.

PLET1 expression in the conceptus: Expression of *PLET1* measured by RT-PCR became stronger during embryo development, [e.g. very weak RT-PCR product was observed in the RT from small spherical concepti after 35 cycles of amplification, while an intense RT-PCR product from filamentous concepti was found after 25 cycles (Fig. 1)]. Expression level of *PLET1* in filamentous concepti appeared to be higher than that observed for *GAPDH*. This is expected as *PLET1* EST were prevalent in libraries made from these tissues. The expression of *Brachyury* increased from

small spherical to large spherical concepti, as found in a previous study[3]; however we could not confirm an increase in *Brachyury* expression from tubular to filamentous concepti (Fig. 1).

Comments: Our linkage mapping data places *PLET1* near *PPP2ARB* and *CRYAB*, but does not enable gene order to be determined. The human locus orthologous to *PLET1* is on chromosome 11q23.2 (LOC349633, LocusLink, NCBI), the evolutionary homologue of SSC9. In human, *PLET1*, PPP2ARB, and *CRYAB* are also tightly linked, with a gene order of *PPP2ARB* (*PPP2R1 beta*, 111,645 Kbp)-*CRYAB* (111,813 Kbp)-*PLET1* (LOC349633, 112,153 Kbp)-*APOA1* (116,740 Kbp). We also found the expression of *PLET1* increased during trophoblastic elongation in the porcine conceptus. The functional role of *PLET1* and factors that regulate its expression are currently unknown.

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DNA sequence, polymorphism, and mapping of luteinizing hormone receptor fragment (*LHCGR*) gene in Great Dane dogs

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Source/description: Exon 11 of the canine luteinizing hormone receptor (*LHCGR*) gene (960 bp) was polymerase chain reaction (PCR) amplified and sequenced. Primers were designed based upon previous bovine *LHCGR* gene sequence (Genbank: 420504). Activating and inactivating mutations have been described in the same receptor in humans.[1]

DNA isolation: Genomic DNA was isolated from Great Dane dogs peripheral blood leucocytes using phenol/chloroform purification based protocols.[2]

Primers sequences:
Primer 1: 5′ TTATTCTGCCATCTTTGCTGAGA 3′
Primer 2: 5′ CTCAGCAACAAAAGAAATCCCT 3′
Genbank accession number: AF389885

PCR conditions: The PCR amplifications were performed in a final volume of 50 μl containing DNA (10 ng), dNTPs 1.25 mM each, *Taq* DNA polymerase (2 UI), *Taq* DNA polymerase buffer 10X, magnesium chloride (2.5 mM) and ultra pure water. Amplification parameters were: 95 °C for 5 min, followed by 30 cycles of 95 °C for 1 min, 55 °C for 30 s, 72 °C for 2 min and one final extension step of 72 °C for 8 min. Negative controls were performed adding all reagents except DNA. All PCR fragments were subsequently submitted to automatic DNA sequencing.

LHCGR: The canine exon 11 fragment shared 89, 89, 92 and 88% identity to the human, bovine, porcine and murine *LHCGR* sequences, respectively.

Sequence analysis: Sequencing of PCR fragments was performed on the ABI 310 (Applied Biosystems®, Norwalk, CT)

Polymorphism: Two polymorphic sites were identified in codons encoding amino acid positions Ile 378 and Val 397 of *LHCGR*. Both polymorphism were silent mutations.

LHCGR isolation of BAC clone containing LHR gene: A canine genomic DNA probe was used for hybridization of canine RPCI 81 BAC/PAC filter (Bacpac Resources/Children's Hospital Oakland Research Institute, Oakland, USA). The 570 bp probe was generated by PCR (Primer 5′ TTATTCTGCCATCTTTGCTGAGA 3′/5′ CCTCCGAGCATGACTGGAATGGC 3′) on genomic DNA of the canine pleomorphic adenoma cell line ZMTH3 (Center for Human Genetics, Bremen, Germany). The obtained PCR product was separated on a 1.5% agarose gel, recovered with QIAEX II (Qiagen, Leusden, Netherlands), cloned in pGEM T-easy vector system (Promega, Madison, USA) and sequenced for verification. The probe labelling was performed by random primed labelling (Roche Diagnostics, Mannheim, Germany) as described in the manufacturer's protocol with 50 μCi(α^{32}P)dCTP (Amersham Pharmacia Biotech, Freiburg, Germany). Purification of the labelled probe was carried out using Sephadex G-50 Nick Columns (Amersham Pharmacia Biotech) and the probe was stored at −20 °C before use. The filters were placed in a minimum volume of Church Buffer (0.15 mM bovine serum albumin, 1 mM ethylenediaminetetraacetic acid, 0.5 M NaHPO4, 7% sodium dodecyl sulphate) and transferred into hybridization bottles. The filters were prehybridized at 65 °C for 1 h in 25 ml Church Buffer. Hybridization was performed at 65 °C overnight (16–18 h) in the same solution. All further steps were performed according to manufacturer's protocol. Signals were visualized using a STORM imager (Molecular Dynamics, Sunnyvale, USA).

Fluorescence in situ hybridization: Metaphase preparations were obtained from blood samples of two different dogs. The samples were stimulated with PHA and cultured for 96 h at 37 °C. After a 1.5 h colcemide (0.1 μg/ml) incubation, the lymphocytes were harvested according to routine procedures. Prior to fluorescence *in situ* hybridization (FISH), chromosomes were stained using the GTG-banding method. After obtaining the

metaphases, the slides were destained in 70% ethanol for 15 min and air dried. FISH was performed using the protocol with some modifications.[3] BAC DNA (RPCI81 272N8) was digoxigenin labelled (Dig-Nick-Translation-Kit; Roche Diagnostics). The hybridization mixture contained 125–175 ng probe, 43.2 μg salmon sperm DNA, 1000–1200 ng sonicated dog DNA, 2× SSC, 2× SSPE, 50% formamide and 10% dextransulphate. The chromosomes were stained with propidium-iodide. Chromosomal G-bands were identified.[4] Canine *LHCGR* was located on chromossome CFA 10.

Acknowledgements: FAPESP – São Paulo – Brasil.

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Identification of two polymorphic microsatellites in a canine BAC clone harbouring a putative canine *MAOA* gene

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Source/description: Monoamine oxidase A (MAOA) is a mitochondrial enzyme which degrades the neurotransmitters noradrenaline and serotonin. Pharmacological and genetic studies suggest that variations in the sequences of the *MAOA* gene could be associated with behavioural changes including aggression and cognitive dysfunction[1]. A canine bacterial artificial chromosome (BAC) library[2] (http://www.dogmap.ch/getclone.htm/) was screened by polymerase chain reaction (PCR) using primers designed to amplify part of the canine *MAOA* exon 15 (AB038563). Subcloning of S048P05H11, subsequent screening and primer design (OLIGO 6.63; Med Probe, Oslo, Norway) yielded the two microsatellites *ZuBeCa57* (F: 5′- ATCCTGAGGTCCTGGTACTGAATC, R: 5′-GTGAAAAA TTAACTGCAAGGTAGGT) and *ZuBeCa61* (F: 5′-GGCTGCTT GGGGGATTTCCTGCTTAAT, R: 5′-TTGTAGAGGACCAGGACG CGCACAC).

PCR conditions: Amplifications were carried out in 12 μl containing 2 μl of DNA solution, 2.5 pmol of each primer, 0·25 mM of each dNTP, 1X PCR buffer with 1.5 mM $MgCl_2$ and 0·35 units *Taq* polymerase (Qbiogene, Basel, Switzerland) in a Perkin-Elmer 9700 thermocycler. PCR was performed using the following touch-down programme[3]: initial denaturation for 5 min at 94 °C, two cycles each of 30 s at 94 °C, 30 s in the respective touch-down range down from the highest to the lowest annealing temperature (63–55 °C) and 30 s at 72 °C, followed by six cycles of 30 s at the lowest annealing temperature. The final extension was for 15 min at 72 °C. Sizes of the alleles were determined on 8% denaturing polyacrylamide gels using a LI-COR DNA sequencer model 4200 (LI-COR, Bad Homburg, Germany).

Nucleotide sequence: GeneBank accession numbers:
ZuBeCa57: AJ604537
ZuBeCa61: AJ604538

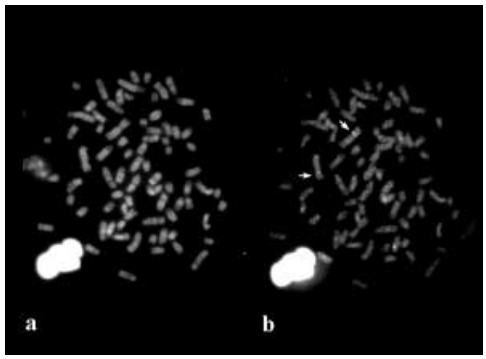

Figure 1 Q-banded metaphase spread of a female dog (a) and the same spread after FISH with the BAC SP048P05H11 (b). The arrows indicate the localization on CFAXp11.

Table 1 Summary data for two canine microsatellites.

	ZuBeCa57 $(TAAA)_{11}$				*ZuBeCa61*$(GT)_{13}$			
	Size range	No. of alleles	PIC	HET	Size range	No. of alleles	PIC	HET
Dog ($n = 24$)	207–223	5	0.49	0.57	83–103	3	0.19	0.21
Red fox ($n = 10$)	203–211	3	0.56	0.71	85–89	3	0.43	0.57
Arctic fox ($n = 10$)	203–215	3	0.18	0.21	85–101	5	0.66	0.78
Chinese raccoon dog ($n = 10$)	199–207	3	0.41	0.54	93–111	4	0.48	0.58

Polymorphism: The size range, number of alleles observed in 24 dogs (*Canis familiaris*) representing 24 different breeds, 10 red foxes (*Vulpes vulpes*), 10 arctic foxes (*Alopex lagopus*) and 10 Chinese raccoon dogs (*Nyctereutes procyonoides procyonoides*), HET and polymorphism information content (PIC) values are given in Table 1. The PIC was calculated according to Botstein *et al.* (1980)[4].

Mendelian inheritance: X-chromosomal inheritance was observed in the DogMap reference family panel[5].

Chromosome location: BAC DNA (S048P05H11), containing the two microsatellites *ZuBeCa57* and *ZuBeCa61* and part of the *MAOA* gene, was labelled with biotin-16-dUTP by random priming and hybridized to Q-banded female dog metaphase chromosome preparations. The clone was localized on chromosome CFAXp11 (Fig. 1) according to the canine partial standard karyotype[6].

Other comments: The 81-bp fragment sequenced from the 109-bp PCR product from the BAC clone S048P05H11 showed 100% identity to putative canine *MAOA* exon 15. A fragment of 578 bp from a 1570-bp contig sequence containing microsatellites *ZuBeCa57* and *ZuBeCa61* showed 78% identity to human *MAOA* intron 10.

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