

BRIEF NOTES

***Tth111I* PCR/RFLP marker in the canine rod transducin alpha (*GNAT1*) gene**

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**Source/description:** PCR primers were designed based on the canine rod transducin alpha cDNA sequence (Genbank accession no. Z69597). A single nucleotide polymorphism (G/A) was found within the amplified sequence. A new primer was designed with a single nucleotide mismatch to create a *Tth111I* restriction site in the presence of the G allele (allele 2), but not the A allele (allele 1). The PCR product from genomic DNA is 397 bp. In the presence of the G allele the PCR product is cleaved into 379 and 18 bp restriction fragments.

**Primer sequences:** Primer 1: 5'-AGAGCACCATCGTCAAGCA-3'

Primer 2: 5'-TGCATCAGCTTCCGGACATC-3'

Primer 2 contains one mismatch (italic) with the canine sequence to create a *Tth111I* restriction site for one allele (allele 2) upon PCR amplification<sup>1</sup>. This polymorphic site is different from the one reported by Ray *et al.*<sup>2</sup>.

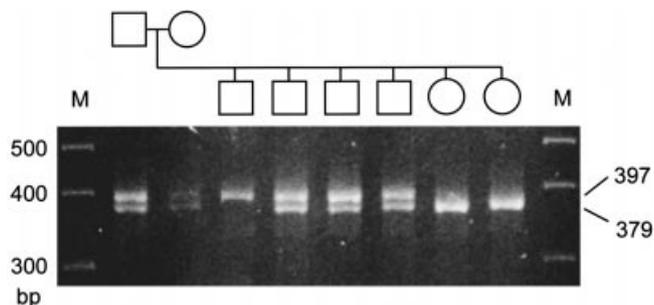
**PCR/RFLP conditions:** PCR was carried out using 50 ng of genomic DNA in a 25- $\mu$ l volume containing the following: 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 100  $\mu$ M dNTPs, 10 pmol of each primer, and 1.25 U of *Taq* polymerase. Reactions were carried out for 35 cycles of 95 °C (1 min), 61 °C (1 min), 72 °C (1 min). The amplification products were precipitated using standard ammonium acetate/ethanol precipitation and 10  $\mu$ g of glycogen as a carrier. Restriction reactions were carried out in a 25- $\mu$ l reaction using 10 U of *Tth111I* in the manufacturer's buffer at 65 °C for 7 h. DNA was visualized on a 4% agarose/1 X TBE gel.

**Allele frequencies:** The relative frequencies of the two alleles were estimated using unrelated dogs of three different breeds; ten American Cocker Spaniels, seven German Shepherd Dogs, and 16 Beagles. The frequencies of alleles 1 and 2 were 0.25 and 0.75, 0.50 and 0.50, and 0.25 and 0.75, respectively. The Beagles and German Shepherd Dogs were from the DogMap Reference Panel<sup>3</sup>. In addition, single animals from ten different breeds were tested to determine the frequencies of alleles 1 and 2. The allele frequencies in these ten animals were 0.50 and 0.50, respectively.

**Chromosomal location:** The chromosomal location of the canine *GNAT1* gene is unknown. The human *GNAT1* gene maps to the short arm of chromosome 3 (3p21)<sup>4</sup>.

**Mendelian Inheritance:** Co-dominant Mendelian inheritance was observed in an eight-member Beagle pedigree (Fig. 1).

**Acknowledgements:** The work was supported by the Glassen Foundation.



**Fig 1.** Demonstration of co-dominant Mendelian Inheritance in an eight-member Beagle Pedigree.

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**Ten equine microsatellite loci: *TKY25*, *TKY26*, *TKY27*, *TKY28*, *TKY29*, *TKY267*, *TKY268*, *TKY269*, *TKY270* and *TKY271***

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**Source/description:** Five trinucleotide (CAG) repeat microsatellites, *TKY25*, *TKY26*, *TKY27*, *TKY28* and *TKY29*, and five dinucleotide (CA) repeat microsatellites, *TKY267*, *TKY268*, *TKY269*, *TKY270* and *TKY271* were characterized. Each trinucleotide repeat locus was isolated from a cosmid clone as described previously<sup>1</sup>. Dinucleotide repeat loci were all obtained using a streptavidin-biotin capture procedure<sup>2</sup>.

**Repeat and PCR primer sequences:**

*TKY25* (CAG)<sub>6</sub>(CAA)<sub>2</sub>(CAG)<sub>3</sub>:

5' AATCTCATGGCAGAATACCG 3'

5' GACTCTGGGAAGGGCTAAGG 3'

*TKY26* (CAG)<sub>11</sub>:

5' AACGCATGCAATTGGCCAG 3'

5' TGCTGTTGCTGCTGGATTGC 3'

*TKY27* (CAG)<sub>3</sub>CAA(CAG)<sub>3</sub>CAA(CAG)<sub>2</sub>:

5' TACTTGGTCCAGCAGCTGTC 3'

5' TCAGAAGCACCTGTCTGTGC 3'

*TKY28* (CNN)<sub>32</sub>(CAG)<sub>12</sub>(CNN)<sub>18</sub>\*:

5' TTCAGCAGGGTCTCATGCCAC 3'

5' TTCGGCTCTGGTTCAAGAGG 3'

*TKY29* (CAG)<sub>5</sub>CAA(CAG)<sub>7</sub>:

5' CTCTTGAACCAGAGCCGAAC 3'

5' TAGAAAAGCGTTGCAGAGGC 3':

*TKY267* (CA)<sub>13</sub>:

5' CCACTGCCAAATGAAACAAA 3'

5' CCACACATTTTCAGGAAAGAA 3'

*TKY268* (CA)<sub>16</sub>:

5' CTGTTATTAGTCTGCCTCTG 3'

5' ACATTCAGACCATCTCAACT 3'

*TKY269* (CA)<sub>15</sub>:

5' ATTTGGGATGTCCTGAACAA 3'

5' TGCATTCAGAGACTTTGTCA 3'

*TKY270* (CA)<sub>16</sub>:

5' CTGCTTTAGAGAAACAAACT 3'

5' CCATGGTGAGAAAAATGAGA 3'

*TKY271* (CA)<sub>21</sub>:

5' CAGTGAAGAGTGAATGGATA 3'

5' GCAAATGGCAGAATTCCTT 3'

\*N indicates A, C, G or T.

**PCR condition:** The PCR amplifications were performed in a total volume of 15  $\mu$ l of the following mixture: 20 ng of horse genomic DNA; 3 pmol of each primer; 333  $\mu$ M of each dNTPs; 25 mM MgCl<sub>2</sub>; 15  $\mu$ l of 10 $\times$  reaction buffer and 1 U of AmpliTaq Gold polymerase (Perkin-Elmer, Foster City, CA). Thermal cycling was carried out in a GeneAmp PCR System 9700 (Perkin-Elmer) with initial denaturation (94 °C for 10 min) and the following cycle parameters: 94 °C for 30 s, 55 °C for 1 min, 72 °C for 30 s for 30 cycles, and final extension for 10 min at 72 °C. PCR products were electrophoresed on an ABI 377 DNA sequencer (Perkin-Elmer).

**Polymorphisms:** Genetic polymorphisms of these loci investigated on Thoroughbred horses are presented in Table 1.

**Table 1** Genetic characteristics of *TKY25–29*, *267–271*

Locus	Tested individual (n)	Allele (n)	Size range (bp)	HTZ	PIC	PE
<i>TKY25</i>	25	1	100			
<i>TKY26</i>	25	2	100–103	0.077	0.074	0.037
<i>TKY27</i>	25	1	160			
<i>TKY28</i>	102	7	276–357	0.705	0.657	0.468
<i>TKY29</i>	25	2	121–127	0.147	0.136	0.068
<i>TKY267</i>	65	4	83–93	0.694	0.642	0.440
<i>TKY268</i>	65	4	178–194	0.530	0.480	0.293
<i>TKY269</i>	65	6	116–128	0.401	0.380	0.231
<i>TKY270*</i>	43	4	167–173	0.679	0.622	0.420
<i>TKY271</i>	65	5	103–127	0.655	0.590	0.387

HTZ, heterozygosity; PIC, polymorphism information content; PE, exclusion probability.

\*TKY270 might be X-linked.

**Chromosomal location:** Chromosomal location of a cosmid clone which include five trinucleotide repeat loci were assigned by FISH based upon the nomenclature of horse chromosomes<sup>3</sup>. Subsequently, *TKY25*, *TKY26*, *TKY27*, *TKY28* and *TKY29* were localized on 10q21.

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## Physical assignment of the porcine erythropoietin receptor gene to SSC2

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*ACP5*, also known as uteroferrin, transports iron from the maternal endometrium to the developing porcine conceptus. Erythropoietin signaling through its receptor (*EPOR*) is critical to erythroid proliferation and differentiation. To further develop the porcine/human comparative map, and in the future identify genomic elements important to the expression of *ACP5* and *EPOR*, large-insert genomic clones containing these genes were isolated from a porcine BAC library. One of the isolated BAC clones was found to contain both genes. Isolated clones were used to physically map the *EPOR* gene to SSC2, in close proximity to *ACP5*.

**Probe synthesis:** <sup>32</sup>P-labeled probes were synthesized by PCR under standard conditions and purified by G-25 sepharose size-exclusion chromatography. The 261-bp *EPOR* probe (base 840–1101, Pearson *et al.*, in preparation) was synthesized with the primers 5'-GCAGAAGATCTGGCCTGG-3' and 5'-GTCTCGGGCATGTTCACTG-3'. The 240-bp *ACP5* probe (base 328–567 GenBank accession M30284, Simmen *et al.* 1989) was synthesized with the primers 5'-GGACAATTCTACTTCACTGGG-3' and 5'-ACAGACACATTG-GACCGTG-3'<sup>5</sup>.

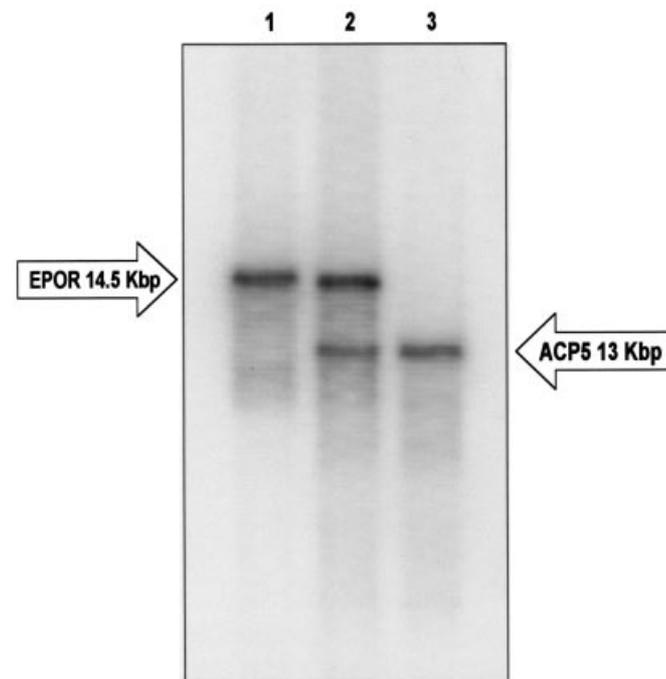


Fig. 1. Porcine BACs 281i21 (1), 324b2 (2), and 339i12 (3) were subject to *EcoRI* digestion, fractionated by agarose gel electrophoresis and capillary transferred to a nylon membrane. The membrane was hybridized with <sup>32</sup>P-labeled *EPOR* and *ACP5* probes and analyzed with the Molecular Dynamics Storm 806 phosphorimager. Sizes were calculated by comparison to a molecular weight ladder ( $\lambda$ bda  $\times$  *HindIII*).

**Filter hybridization:** High-density BAC filters representing 1/2 ( $\approx$  5 genome equivalents) of the RPCI-44 Male Porcine BAC library (BACPAC Resources, Buffalo, NY) were hybridized overnight with  $1 \times 10^6$  cpm of each probe in 10 ml hybridization solution, washed and exposed to X-ray film. Identified BACs were grown in LB broth at 15  $\mu$ g/ml chloramphenicol and purified by standard alkaline lysis<sup>13</sup>. Approximately 500 ng of each BAC was subjected to restriction digestion with 2 units of *EcoRI* for 3 h at 37 °C, fractionated on a 0.5% TBE-agarose gel and transferred to nylon membranes by denaturing capillary transfer. The membranes were then hybridized with  $1 \times 10^6$  cpm/ml *EPOR* and *ACP5* probes in RapidHyb (Amersham) for 1 h at 65 °C, washed once with 1X SET, exposed to a storage phosphor screen (Kodak) overnight, and analyzed on a phosphorimager (Molecular Dynamics Storm 860).

**FISH:** The FISH protocol was as described by Lopez-Corrales and coworkers<sup>11</sup> with the exception that 500 ng of isolated BAC DNA was labeled with biotin by nick-translation (Bionick, LTI) and hybridized to porcine metaphase spreads.

A single BAC clone with an estimated 103 Kb genomic DNA insert was identified that contained both the *EPOR* and *ACP5* genes. This BAC, as well as BACs containing *EPOR* or *ACP5* exclusively, were subjected to restriction analysis and Southern

hybridization. The *EPOR* probe hybridized to a  $\approx 14.5$  Kb *EcoRI* fragment in BAC clones containing both genes or *EPOR* exclusively. The *ACP5* probe hybridized to a  $\approx 13$  Kb *EcoRI* fragment in BAC clones containing both genes or *ACP5* exclusively (Fig. 1). This supports the specificity of the probe hybridizations and the close physical proximity of these two genes in BAC 324b2. To rule out the possibility that the proximity of these two genes in this BAC clone is due to chimerism, BAC clones containing *EPOR* or *ACP5* exclusively were used as probes for FISH analysis. As presented in Fig. 2, the *EPOR* clone hybridized to SSC2q1.2-2.1. Fish analysis of the *ACP5* clone yielded similar results. Both probes consistently hybridized to a single region of the porcine genome, SSC2q1.2-2.1. These results physically map the erythropoietin receptor gene to SSC2, in close proximity to *ACP5*.

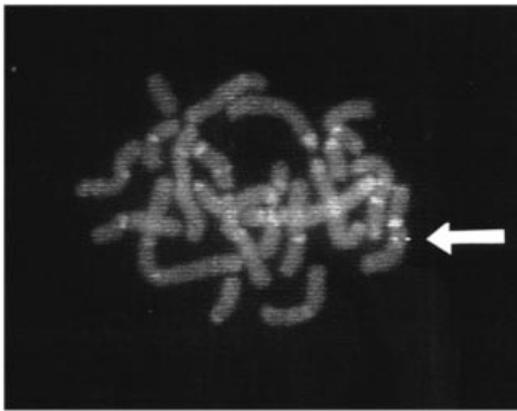


Fig. 2. A partial metaphase spread showing porcine BAC 281i21, containing the *EPOR* gene, hybridization (signals designated by arrows) to porcine SSC2q.

We have physically assigned the porcine *EPOR* gene to SSC2, within 103 Kb of the *ACP5* gene which had previously been assigned to this location<sup>9,15</sup>. The proximity of these genes reflects the conservation of synteny of this chromosomal segment in mice<sup>2,7</sup>, humans and pigs<sup>12,4,6</sup>. The human *ACP5* and *EPOR* genes are also likely to be very close, supported by their being mapped to within 0.18 cR<sub>3000</sub> of each other with the Stanford Genebridge4 radiation hybrid panel<sup>3</sup>. The sensitivity of erythropoiesis, as well as erythropoietin and *EPOR* expression, to iron availability and oxidation-state is well established<sup>5,8,10</sup>. The expression of human *ACP5* is also sensitive to the availability of iron<sup>1</sup>. Importance to erythropoiesis, mutual sensitivity to iron concentration and close physical proximity of these two genes presents the possibility that their expression may in some way be coordinated. However, these genes are predominantly expressed in different tissues<sup>14</sup> (Pearson *et al.*, in preparation). Future characterization of the sequences surrounding these genes and the factors which regulate their expression in pigs will address this prospect.

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## Identification of a *MaeI* RFLP in the insulin-like growth factor-1 (*IGF1*) gene of swamp buffaloes (*Bubalus b. bubalis kerebau*)

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**Source/description:** The insulin-like growth factors (IGF1 and IGF2) play an important role in the regulation of growth, development and metabolism in mammals. IGF1 is an essential mediator of normal postnatal growth and its expression is influenced by hormonal, nutritional, tissue-specific and development factors. Genetic correlations to phenotypic measurements of circulation blood serum IGF1 of hepatic and non-hepatic IGF1 synthesis have been described<sup>1</sup>. The bovine insulin-like growth factor-1 gene (*IGF1*) was mapped to the linkage group U3 on BTA5<sup>2</sup>. Here we describe the identification of a polymorphism within the *IGF1* gene of swamp buffaloes (*Bubalus b. bubalis kerebau*;  $2n = 48$ ). We have amplified a 1394-bp fragment of the *IGF1* gene harbouring parts of exon 4 and intron 3. Within intron 3 a C→A transversion polymorphism was identified that alters a *MaeI* site. The DNA sequence has been deposited with the EMBL DNA Sequence Database under accession number Y18832.

#### Primer sequences:

Forward: 5'-TTC TCC CTT CCA CTA CTG TG-3'

Reverse: 5'-TCT CCT TCT GTT CCC CTC-3'

**PCR conditions:** PCR primers were designed on the basis of the published sequence of the bovine *IGF1* gene<sup>3</sup> (EMBL acc. no. U01338) at positions 215–234 (forward) and 640–657 (reverse). PCR (25  $\mu$ l final volume) was performed using 100 ng of genomic DNA, 10 mM Tris-HCl pH 8.85, 25 mM KCl, 5 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2 mM MgSO<sub>4</sub>, 0.2 mM dNTP, 20 pM of each primer and 1 U *Taq* DNA polymerase. The PCR conditions were 33 cycles of 60 s at 94 °C, 60 s at 55 °C and 90 s at 72 °C with an additional 3 min denaturation in the first cycle and 5 min extension in the last cycle using a TECHNE thermocycler (Cambridge, UK). PCR products were purified with the QIAEX DNA Gel-Extraction Kit (QIAGEN, Hilden, Germany) and analysed using the ThermoSequenase Fluorescence Labelled Cycle Sequencing Kit (Amersham-Pharmacia Biotech, Freiburg, Germany) and a DNA sequencer LI-COR 4000L (MWG Biotech, Ebersberg, Germany). The PCR products were digested with 2 U of *MaeI* at 45 °C for 2 h. This was followed by electrophoresis in a 2% agarose gel stained with ethidium bromide to visualise the restriction fragments.

**Polymorphism:** A single nucleotide polymorphism (SNP) was detected at position 564 of the amplified fragment within intron 3. The digested PCR product revealed two alleles with fragment sizes for allele A of 651 bp; for allele B fragment sizes were 469 and 182 bp (Fig. 1). Five constant bands at 620, 61, 34, and 28 bp were also detected. The polymorphism was only detected in individuals of *Bubalus b. bubalis kerebau*.

**Chromosomal location:** The chromosomal location of the *IGF1* gene of *Bubalus b. bubalis* is currently unknown.

**Mendelian inheritance:** Mendelian inheritance was followed in 24 individuals of 11 families of *Bubalus b. bubalis*.

**Frequency:** A total of 65 Brazilian swamp buffaloes were genotyped using the *MaeI* RFLP and allele frequencies were estimated to be 0.53 for allele A and 0.47 for allele B.

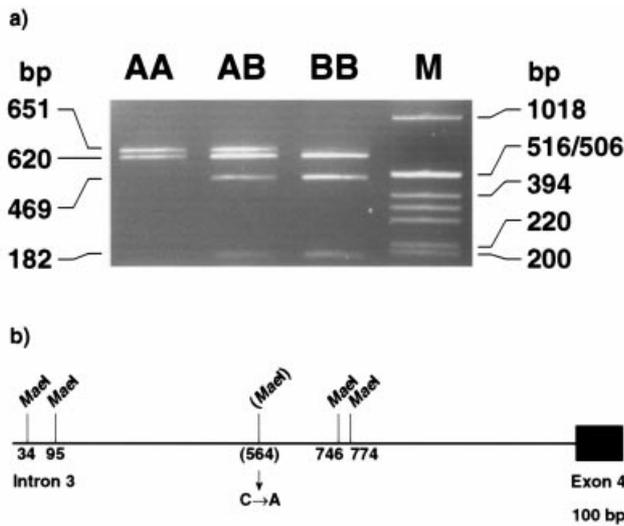


Fig. 1. Partial genomic structure and *MaeI* polymorphism in intron 3 of the *IGF1* gene of *Bubalus b. bubalis kerebau*. (a) The 1394 bp *IGF1* gene fragments were amplified as described, digested with *MaeI* and separated on a 2% agarose gel. Products were visualised after ethidium bromide staining. The genotypes of the different animals are shown at the top of each lane (AA, AB, BB). Sizes are indicated in bp on the left side. The right lane (M) shows the fragments of a 1-kb DNA ladder (GIBCO BRL, Eggenstein, Germany). (b) The diagram outlines the partial genomic structure of the *IGF1* gene including parts of intron 3 and exon 4 as amplified by PCR. The *MaeI* sites are shown with their positions (numbers below the diagram).

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## A DNA polymorphism in the bovine *c-kit* gene

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**Source/description:** Primers for amplification and sequencing of bovine intron sequences were constructed based on both the bovine *c-kit* cDNA sequence<sup>1</sup>, and exon-intron positions in the corresponding human gene<sup>2</sup>. The RFLP primer positioned within intron 3 were designed from intron 3 sequence data.

**Primer sequences (5'-3'):**

Exon 3 forward primer:

TGTAAAACGACGCGCCAGTATGTGAAGCGCAGTACCAT

Exon 4 reverse primer: CCACGGAAGTAGACACGTCT

Forward-RFLP primer (intron 3): TAATTACTTGGGACGCATAG

Reverse-RFLP primer (exon 4): TTTTGGACACAGACACAAC

**PCR conditions:** Amplification of intron 3 was carried out in an 10- $\mu$ l reaction containing 10 pmol of each primer (exon 3 forward primer and exon 4 reverse primer), 0.5 U *Taq* polymerase, standard buffer (Perkin Elmer, NJ, USA), 0.2 mM of each dNTP, and 50 ng DNA. Genomic DNA was denatured for 3 min at 95 °C, and PCR run for 40 cycles at 95 °C for 15 s, 62 °C for 30 s, and 73 °C for 60 s. RFLP-PCR was carried out in a similar reaction as described above

using forward and reverse RFLP-primers. Genomic DNA was denatured for 3 min at 95 °C, and PCR run for 40 cycles at 95 °C for 15 s, 60 °C for 15 s, and 73 °C for 30 s.

**Sequencing:** Sequencing of intron 3 from the bovine *c-kit* gene were done directly from the PCR product using dye primer chemistry (Perkin Elmer). The sequence contained an A to C substitution resulting in a CCTAA sequence being recognised by the restriction enzyme *MnII* (accession number AJ243424 (allele 1) and AJ243060 (allele 2)).

**Polymorphism:** For allele 1, the 221 bp PCR product is cut by *MnII* yielding fragments of 65 and 156 bp. In allele 2, the 156 bp fragment is further digested to fragments of 108 bp and 48 bp. Fragments were resolved on a 3% agarose gel. Among 6 half sib families used in the construction of the Norwegian Cattle Map (NCM)<sup>3</sup>, two heterozygous sires with 125 sons were genotyped.

**Allele frequency:** Allele frequencies were estimated in two paternal half-sib families of norwegian Cattle (NRF) exclusively utilising alleles inherited from the dams of 125 sons. The allele frequencies of allele 1 and allele 2 (allele being recognised by *MnII*) were 0.62 and 0.38, respectively. Additionally, the polymorphism has been tested in Icelandic cattle, 6 local Norwegian cattle breeds and an African cattle breed (the long-horned Ankole from Uganda). Both alleles were present in all these breeds.

**Chromosomal location:** Two-point linkage analysis detected linkage to markers on bovine chromosome 6, which is in accordance with previous mapping data<sup>4</sup>. No recombinants were observed with marker *RM28*, resulting in a distance of 0.0 cM (lod score 33.3) to this marker. Distances to other closely mapped loci were (lod scores in brackets): *FBN13*-6.0 cM (12.7)-*KIT*-16.9 cM (5.9)-*BP7*.

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## Variable microsatellites in the Pacific Oyster *Crassostrea gigas* and other cupped oyster species

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**Source/description:** Genomic DNA was extracted from a whole Pacific oyster (*Crassostrea gigas*) after grinding in liquid nitrogen. Purified DNA was then digested by a mix of three restriction enzymes (*AluI*, *RsaI*, *HaeIII*). DNA fragments ranging from 250 to 500 bp were size-selected by agarose gel electrophoresis and ligated into the dephosphorylated blunt-ended *SmaI*-linearised pBKS2 plasmid (Stratagene Cloning System, La Jolla, CA). White colonies (11 500) were screened by hybridisation with double-stranded DNA probes containing dinucleotide repeats. Poly(dAdC), poly(dAdG), poly(dAdT) (Pharmacia Biotech, Uppsala, Sweden) were mixed at equimolar ratio and radiolabelled by [ $\alpha$ -<sup>32</sup>P] alpha dATP and dCTP using a random priming labelling kit (Life Technologies, Germany). Filter hybridisation and subsequent washing were carried out at 65 °C in 5 $\times$  SSC. Fifty-two positive clones were identified with dinucleotide microsatellites. Plasmid DNA from minipreps performed on these positive clones were cut in the multicloning site of pBKS2 in order to determine the insert length. Plasmid DNA was prepared and sequenced using forward and reverse primers present on each side of the pBKS2 multicloning site. Only six positive clones contained a microsatellite motif out of the 51 sequenced. Specific primers were designed for 5 of them.

**PCR reaction:** Radioactive PCR amplification was carried out in a total volume of 10  $\mu$ l using 2  $\mu$ l of extract containing oyster genomic DNA. Reagents included dNTPs (0.074 mM), 1 pmol of each primer, and 0.35 U of *Taq* polymerase (Promega, Madison, WI). Note that the MgCl<sub>2</sub> concentration was optimised for each marker (Table 1). Radioactive labelling of the amplification fragment was carried out using 0.25 pmol of a primer phosphorylated with [ $\gamma$ -<sup>32</sup>P]-ATP. After a denaturing step of 1 min at 94 °C, samples were processed through 30 cycles consisting of 1 min at 94 °C, 1 min at the optimal annealing temperature (Table 1) and 1 min 15 s at 72 °C. The last elongation step was lengthened to 5 min.

**Polymorphism:** Four loci were found to be polymorphic with the primer pairs designed and exhibited a high variability (Table 1). One of the loci (*L10*) was scored on 324 individuals *C. gigas* from different geographical origins and displayed 49 alleles. The allelic PCR products differed from each other in multiples of 2 base pairs.

**Cross-species amplification:** We also examined the conservation of the four polymorphic loci in ten species of the genus *Crassostrea*

and two of the genus *Saccostrea* (Table 2). PCR amplifications were performed using the same reaction conditions as above. These microsatellite loci were also amplified in closely related Asian taxa: *C. angulata*, *C. sikamea* and *C. ariakensis*, three cupped oysters of interest in aquaculture. These fragments were not sequenced but it is likely that homologous loci were amplified in these species, as they showed similar size and similar allelic variation as those obtained for *C. gigas*. In addition, the primers for the *L48* locus also allowed the amplification of specific products from *C. gasar*, *C. rhizophorae*, *S. cucullata* and *S. commercialis* but the yields of amplified DNA were too low to allow the identification of allelic variation.

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**Table 1.** Characterisation of four polymorphic microsatellite loci in the Pacific cupped oyster *C. gigas*. Ta is the optimal annealing temperature, He is the expected heterozygosity and Ho the observed heterozygosity. Sample size was 17 individuals for all loci, except for the *L10* locus where it was 468 individuals

Locus	Repeat array	Primer sequences (5'–3')	GenBank accession number	Annealing temperature (°C)	[MgCl <sub>2</sub> ] (mM)	Allele number	Mean size		range of PCR product (bp)
							Ho	He	
<i>L8</i>	(AG) <sub>22</sub>	AGAGGTTCAATGACGCTGGTG GATAAACAGTTTTCTGGTGTTAC	AF170849	57	1	14	0.54	0.85	181
<i>L10</i>	(AG) <sub>26</sub>	GGTCAATTCAAAGTCAATTTCCC CATGTTTTCCCTTGACTGATCC	AF170850	55	1	49	0.90	0.96	136
<i>L16</i>	(AG) <sub>24</sub>	CGGACGAATAAGATATTTGGTC TGGATCTGCGCATCATCTCG	AF170851	57	1	15	0.64	0.83	164
<i>L48</i>	(GA) <sub>n&gt;30</sub>	TCAAACCATCTGCTCGTCTACG TCCGAAAATCCAGGAATACCGG	AF170852	60	1.5	13	0.73	0.87	161

**Table 2.** Cross-species amplification for four microsatellite loci

Species	N	Locus			
		<i>L8</i>	<i>L10</i>	<i>L16</i>	<i>L48</i>
<i>Crassostrea angulata</i>	10	+	+	+	+
<i>Crassostrea ariakensis</i>	5	–	1 + of 5 tested	+	+
<i>Crassostrea sikamea</i>	5	–	+	+	+
<i>Crassostrea virginica</i>	5	–	–	1 + of 5 tested	+
<i>Crassostrea iredalei</i>	1	–	–	–	–
<i>Crassostrea belcheria</i>	1	–	–	–	–
<i>Crassostrea margaritacea</i>	3	–	–	–	–
<i>Crassostrea echinata</i>	5	–	–	–	–
<i>Crassostrea gasar</i>	5	–	–	–	?
<i>Crassostrea rhizophorae</i>	4	–	–	–	?
<i>Sassostrea cucullata</i>	5	–	–	–	?
<i>Sassostrea commercialis</i>	4	–	–	–	?

+, amplification; –, no amplification; ?, slight amplification without reading the genotypes.

## Four microsatellite markers in the Japanese flounder, *Paralichthys olivaceus*

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**Source and description:** A size selected genomic library (300–500 bp) was constructed using DNA obtained from Japanese flounder, *Paralichthys olivaceus*, digested with *Sau3AI*. The DNA was inserted in pBluescriptSK digested with *Bam*H1 and transformed into competent *Escherichia coli* cells (strain DH5 $\alpha$ ). The partial genomic library was screened with a  $\gamma$ -<sup>32</sup>P labeled (CA)<sub>10</sub> probe. Sequencing of positive clones was carried out on ABI Prism 310 Genetic analyzer using BigDye Terminator cycle sequencing kit (Perkin Elmer Japan Applied Biosystems, Tokyo, Japan). Primers flanking the dinucleotide repeats were designed.

**PCR conditions:** PCR was performed in a 25- $\mu$ l solution containing 12.5 pmol of forward primer and 0.6 pmol of reverse primer end-labeled with  $\gamma$ -<sup>32</sup>P ATP, 0.6 units of *Taq* Polymerase (Takara, Tokyo, Japan) 1  $\times$  PCR buffer (50 mM of KCl, 10 mM of Tris-HCl, pH 8.3, 1.5 mM of MgCl<sub>2</sub>), 50 ng of genomic DNA, 200  $\mu$ M of each dNTP and

**Table 1.** Characterization of four flounder microsatellites

Name	Primer sequence	Repeat	Annealing temperature (°C)	Unrelated individuals screened (n)	Number of alleles	Size range (bp)	H	PIC	Genbank access number
<i>Poli-RC04-TUF</i>	F5'GATCTTGCGAACTGGTCTCATGG R5'GCGGCAAGATCAAATAACAGGTTCT	(CA) <sub>9</sub>	65	22	5	118–125	0.64	0.68	AB030935
<i>Poli-RC12-TUF</i>	F5'AGGATGCAGGCTGCTTTG R5'ATGTCCTTTTTTGCCATTTG	(CA) <sub>29</sub>	62	21	25	102–209	0.81	0.95	AB030936
<i>Poli-RC27-TUF</i>	F5'CCAATGCATCAATACGTACACA R5'ACCAGCTGCCCTTCATACAC	(CA) <sub>24</sub>	62	22	19	77–136	0.91	0.90	AB030937
<i>Poli-RC47-TUF</i>	F5'TGACCAGACGCACTGAGTTC R5'TCTGCTTCACTGACAAAAACA	(CA) <sub>26</sub>	62	22	12	59–104	0.91	0.85	AB030938

F, Forward; R, Reverse; H, Heterozygosity; PIC, Polymorphism Information Content.

1% BSA. Thermal cycling was performed in a GeneAmp PCR System 9600 (Perkin Elmer Japan Applied Biosystems, Tokyo, Japan) and consisted of an initial denaturation at 95 °C for 2 min, followed by 35 cycles of 95 °C for 30 s, 1 min at the annealing temperature, 72 °C for 1 min and a final extension of 3 min at 72 °C. PCR products were separated in a 6% polyacrylamide gel. Allele sizes were determined using a M13 standard sequencing ladder.

**Mendelian inheritance:** Codominant Mendelian inheritance of alleles was confirmed for the four microsatellite loci in a family of farmed flounder.

**Polymorphism:** Genomic DNA taken from unrelated flounders caught in three different areas off Japanese coast were analysed to estimate the number of alleles and PIC (Table 1).

**Chromosomal location:** Unknown.

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## Two bovine dinucleotide repeat polymorphisms: *RM084* and *RM171*

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**Source/description:** The microsatellites *RM084* and *RM171* were isolated from a library of size-selected (≈ 300 bp) *Mbo*I restriction fragments of bovine DNA ligated into M13mp18 and screened with an end-labeled (CA)<sub>10</sub> oligonucleotide probe. Sequences were obtained by conventional dideoxy methods and primers were synthesized to amplify the repeat regions.

**Primer sequences (5'–3'):**

*RM084* (CA strand): GGGAGGGGAAATCTTTGCATTC

(GT strand): GTGACTGGAGGTCTCAGCCT

*RM171* (CA strand): AGTATTGGAGCTTAGTTCA

(GT strand): TGGATGTGACTTAGAAAC

**PCR conditions:** Reactions were performed exactly as described by Kossarek *et al.* 1994<sup>1</sup>. Amplification products were analyzed by electrophoresis in denaturing 6% polyacrylamide gels followed by autoradiography.

**Polymorphism:** Polymorphism was observed with both markers in the parents of the MARC reference families<sup>2</sup>. Data are summarized in Table 1.

**Mendelian inheritance:** Mendelian segregation of alleles was observed in informative pedigrees of the MARC reference families<sup>2</sup>.

**Chromosomal location:** Markers *RM084* and *RM171* have been assigned to bovine chromosomes 5 and 2, respectively, by linkage analysis (CRI-MAP version 2.4) in the MARC reference mapping population.

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## Four highly polymorphic dinucleotide microsatellites in rainbow trout (*Oncorhynchus mykiss*)

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**Source/description:** The microsatellites *OmyRGT10TUF*,

**Table 1.** Characterization of *RM084* and *RM171*

Marker	GenBank access. no.	No. alleles (inf. meioses)	Size range (bp)	Annealing temperature (°C)	Two-point linkage analysis*		
					LOD	Rec. frac.	Marker
<i>RM084</i>	U32916	6 (279)	130–140	54	62.81	0.00	<i>BMS1617</i>
<i>RM171</i>	AF160184	7 (225)	141–165	58	48.30	0.03	<i>BMS778</i>

\*Data presented only for the highest LOD score.

*OmyRGT34TUF*, *OmyRGT47TUF* and *OmyRGT55TUF* were isolated from a plasmid library of size-selected (150–400 bp) *Sau3AI* restriction fragments from the RTG-2 cell line<sup>1</sup> by screening with an end-labelled (CA)<sub>10</sub> oligonucleotide probe. The sequence was obtained from an ABI 310 automated sequencer. PCR primers designed on the basis of the flanking sequences are described in Table 1.

**PCR conditions:** The PCR amplification (25 µl final volume) was performed using 50 ng of genomic DNA, 20 pmol of each primer, 0.2 U of *Taq* polymerase (TaKaRa, Shiga, Japan), 200 mM each dNTP, 1.5 mM MgCl<sub>2</sub> and PCR buffer (Tris-HCl, 10 mM; KCl, 50 mM; pH 8.3). The thermal cycler profile was 2 min at 95 °C followed by 35 cycles of 95 °C for 30 s, 58 °C for 1 min, 72 °C for 1 min and final extension of 3 min at 72 °C. Products were analysed by electrophoresis in 6% denaturing polyacrylamide gels followed by image reading on a Bio-Imaging Analyser (Fujix, Tokyo, Japan).

**Polymorphism:** Polymorphisms were studied in 17 unrelated rain-

bow trout obtained from five Japanese hatcheries. Information regarding the designated markers is shown in Table 1.

**Mendelian inheritance:** Mendelian inheritance was confirmed by typing five half-sib families.

**Chromosomal location:** Unknown.

**Comments:** The sequences of *OmyRGT10TUF*, *OmyRGT34TUF*, *OmyRGT47TUF* and *OmyRGT55TUF* have been submitted to DDBJ/EMBL/GenBank (accession numbers: AB031198, AB031199, AB031200 and AB031201, respectively).

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**Table 1.** Summary data for the four new microsatellite markers in rainbow trout

Marker	Primer sequence (5'–3')	Repeat	Alleles (n)	Size range (bp)	Heterozygosity	PIC
<i>OmyRGT10TUF</i>	F: TGAAAGTCATGTCAGAGTGCTC R: CAATCTCAGAATTCACCCAGC	(CA) <sub>53</sub>	12	84–183	0.76	0.75
<i>OmyRGT34TUF</i>	F: CTTATTTGCTTTGGCACAAGC R: CTCCTCTCCCTTTCTGTCTACCG	(CA) <sub>5</sub> CT(CA) <sub>29</sub>	14	91–179	0.87	0.86
<i>OmyRGT47TUF</i>	F: GACCAAGAGGACCAACGGTA R: ATCTCCGTGCAGTCTCTCG	(CA) <sub>7</sub> TA(CA) <sub>11</sub>	9	95–147	0.76	0.74
<i>OmyRGT55TUF</i>	F: CGTTTTATCCGGTGCCAG R: CACGTCCAACAATATGGTGC	(CA) <sub>10</sub> TA(CA) <sub>21</sub>	7	83–121	0.69	0.65

## A diallelic short tandem repeat (CCCCG)<sub>4</sub> or <sub>5</sub>, located in intron 1 of rabbit α-globin gene

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**Source/description:** PCR primers prepared from published sequences of the rabbit α-globin gene<sup>1</sup> (GenBank accession number M35026) were used to amplify a 368-bp DNA segment comprising exon 1, intron 1 and part of exon 2. This segment included a (CCCCG)<sub>4</sub> repeat located in intron 1 and found to be a protein binding site that is probably implicated in the regulation of the *HBA* gene expression<sup>2</sup>. Following amplification, the PCR products were digested with *Sau96I*, separated on a T9C5 native polyacrylamide gel and silver stained.

#### Primer sequences:

Primer 1: 5'-TGGGCTCCGCACACTTCTGG (M35026 nt 6733–6752)

Primer 2: 5'-GACAGGGCGCCGGGCAGGTCGTCC (M35026 nt 7100–7077)

**PCR conditions:** PCR was performed in a total volume of 25 µl of the following mixture: 20 ng of rabbit genomic DNA; 1 µM of each primer; 200 µM of each dNTPs; 1.5 mM MgCl<sub>2</sub>; 2.5 µl 10× PCR buffer (0.75 M Tris-HCl pH 9.0, 0.2 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.1% w/v Tween (20); 10% DMSO and 0.5 units of *Taq* polymerase. Amplification was carried out using a Perkin Elmer thermal cycler (Perkin Elmer, Foster City, CA) with the following cycling conditions: initial denaturation of 10 min at 94 °C, followed by 40 cycles of 1 min each at 94 °C, 62 °C and 72 °C, and a final elongation of 10 min at 72 °C.

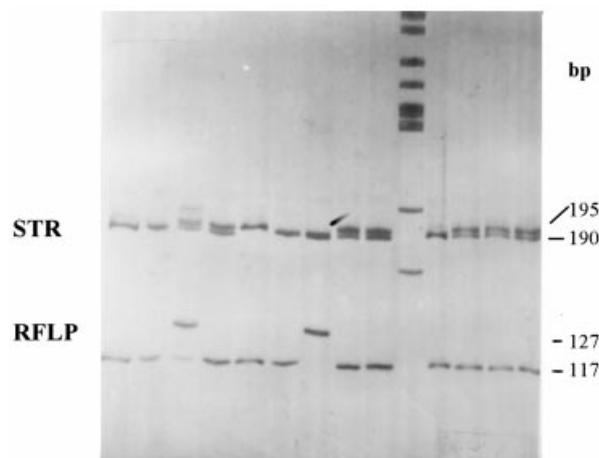
**Polymorphism:** In a survey of 51 domestic rabbits representing four breeds, 95 of the chromosomes (93%) had the (CCCCG)<sub>4</sub> allele and seven of the chromosomes (7%) had the (CCCCG)<sub>5</sub> allele. A sample of 76 portuguese wild rabbits showed 55 chromosomes (36%) with

the (CCCCG)<sub>4</sub> allele and 97 chromosomes (64%) with the (CCCCG)<sub>5</sub> allele. The nature of this polymorphism was confirmed by direct sequencing of PCR products.

**Chromosomal location:** The *HBA* locus has been mapped to chromosome 6q12<sup>3</sup>.

**Other comments:** The use of *Sau96I* allowed the simultaneous detection of a RFLP (Fig. 1) due to a G → C substitution (nucleotide 6866 of GenBank accession number M35026) that is responsible for the aminoacid polymorphic position α29 (Val/Leu)<sup>4,5</sup>.

**Acknowledgements:** Profs Ross Hardison and J.B. Clegg provided helpful suggestions concerning the PCR conditions. This work was partially supported by a grant from Direcção-Geral das Florestas, and by project GENRES 060. N Ferrand and M Azevedo are supported by



**Fig. 1.** Silver-stained 9% polyacrylamide gel showing (i) a diallelic STR polymorphism, and (ii) a *Sau96I* PCR/RFLP, in rabbit *HBA* gene.

FCT grants (FMRH/BSAB/50/98 and PRAXIS XXI/BTI/12139/97, respectively).

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## South American camelid microsatellite amplification in *Camelus dromedarius*

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**Description:** In recent publications a total of 37 polymorphic microsatellite primer pairs for South American camelids have been reported<sup>1–3</sup>. These microsatellites were tested for cross-species amplification in *Camelus dromedarius*. According to our results 9 out of 15 YWLL<sup>1</sup>, 8 out of 12 LCA<sup>2</sup> and 7 out of 10 VOLP<sup>3</sup> (a total of 24) primer pairs amplified in *Camelus dromedarius* genomic DNA. The same markers also amplified in one Tulu (paternal *C. bactrianus* & maternal *C. dromedarius*) and therefore, should amplify in *Camelus bactrianus*.

**Table 1.** Characteristics of 17 South American camelid microsatellites amplified in dromedary racing camels

Name	Alleles (n)	Size range (bp)	H	PIC	P <sub>E</sub>
YWLL02	6	290–304	0.797	0.768	0.603
YWLL08	12	134–172	0.819	0.796	0.647
YWLL29	2	206–208	0.273	0.236	0.118
YWLL38	3	182–188	0.609	0.541	0.332
YWLL44	6	090–114	0.703	0.652	0.454
YWLL59	2	107–109	0.497	0.374	0.187
LCA5	3	165–169	0.038	0.038	0.019
LCA8	2	232–234	0.453	0.350	0.175
LCA18	3	224–230	0.642	0.568	0.351
LCA30	2	218–220	0.495	0.373	0.186
LCA33	7	136–164	0.722	0.676	0.486
LCA37	2	132–133	0.233	0.206	0.103
VOLP03	8	144–176	0.629	0.590	0.407
VOLP08	3	144–148	0.511	0.427	0.239
VOLP10	6	249–267	0.741	0.701	0.517
VOLP32	3	256–262	0.235	0.212	0.109
VOLP67†	19	149–203	0.871	0.858	

†The marker was X-linked.

**Table 2.** Allelic frequencies for 12 South American camelid microsatellites that amplified three or more alleles in *Camelus dromedarius*

YWLL02	YWLL08	YWLL38	YWLL44	LCA5	LCA18						
<b>290</b>	0.21	<b>134</b>	0.19	<b>182</b>	0.24	<b>090</b>	0.01	<b>165</b>	0.01	<b>224</b>	0.22
<b>294</b>	0.12	<b>140</b>	0.01	<b>184</b>	0.23	<b>104</b>	0.10	<b>167</b>	0.98	<b>226</b>	0.44
<b>298</b>	0.17	<b>146</b>	0.29	<b>188</b>	0.53	<b>106</b>	0.41	<b>169</b>	0.01	<b>230</b>	0.34
<b>300</b>	0.31	<b>148</b>	0.02			<b>108</b>	0.29				
<b>302</b>	0.13	<b>152</b>	0.01			<b>110</b>	0.18				
<b>304</b>	0.07	<b>156</b>	0.01			<b>114</b>	0.01				
		<b>160</b>	0.18								
		<b>162</b>	0.04								
		<b>164</b>	0.11								
		<b>166</b>	0.12								
		<b>168</b>	0.02								
		<b>172</b>	0.01								
LCA33	VOLP03	VOLP08	VOLP10	VOLP32	VOLP67						
<b>136</b>	0.14	<b>144</b>	0.56	<b>144</b>	0.62	<b>249</b>	0.38	<b>256</b>	0.01	<b>149</b>	0.18
<b>145</b>	0.35	<b>146</b>	0.03	<b>146</b>	0.33	<b>251</b>	0.13	<b>260</b>	0.13	<b>151</b>	0.01
<b>147</b>	0.36	<b>154</b>	0.01	<b>148</b>	0.06	<b>259</b>	0.27	<b>262</b>	0.87	<b>153</b>	0.19
<b>149</b>	0.03	<b>162</b>	0.04			<b>261</b>	0.13			<b>155</b>	0.19
<b>152</b>	0.10	<b>164</b>	0.21			<b>265</b>	0.09			<b>157</b>	0.01
<b>162</b>	0.02	<b>166</b>	0.04			<b>267</b>	0.01			<b>159</b>	0.01
<b>164</b>	0.01	<b>168</b>	0.11							<b>173</b>	0.02
		<b>176</b>	0.01							<b>175</b>	0.01
										<b>177</b>	0.09
										<b>179</b>	0.09
										<b>181</b>	0.04
										<b>183</b>	0.01
										<b>185</b>	0.01
										<b>187</b>	0.05
										<b>189</b>	0.03
										<b>191</b>	0.03
										<b>193</b>	0.02
										<b>195</b>	0.01
										<b>203</b>	0.01

Allele sizes are in bold type numbers.

**PCR conditions:** The PCR conditions used were according to the published parameters<sup>1-3</sup>, except that *AmpliTaq* Gold (Perkin-Elmer, Germany) and Platinum Taq (GibcoBRL-Life Technologies, Germany) were used interchangeably. For markers *YWLL38*, *LCA18* and *VOLP03* Platinum GenoType Tsp (GibcoBRL, Life-Technologies, Germany) was used to increase the clarity of the banding pattern. PCR reactions contained 100 ng of DNA, 0.2 mM each dNTP, 1 × PCR buffer, 1.5 mM MgCl<sub>2</sub>, 0.25 μM for each primer and 1.0 U of DNA polymerase in a final volume of 15 μl. Forward primers were dye labelled with IRD 800 (MWG Biotech, Germany). PCR reactions were carried out in a Hybaid Touchdown (Hybaid-AGS, Germany) thermal cycler. PCR products were electrophoresed on a LiCor 4000L DNA sequencer (MWG Biotech, Germany). Allele sizes were determined with RFLPscan Plus Version 3.0 (Scanalytics, A division of CSPi).

**Polymorphism:** Polymorphism of the 24 markers which amplified were evaluated in a group of 52 unrelated dromedary racing camels (*Camelus dromedarius*). *LCA19* & *LCA24*, *VOLP12* & *VOLP33* and *YWLL36*, *YWLL40* & *YWLL46* (a total of seven markers) proved to be monomorphic across a panel of 44 camels out of the above 52 (eight were not done). Characteristics of the remaining 17 microsatellites are presented in Table 1 with heterozygosity (H)<sup>4</sup>, polymorphic information content (PIC)<sup>5</sup> and probability of exclusion (P<sub>E</sub>)<sup>6</sup> values. Allele sizes and frequencies of markers with three or more alleles are presented in Table 2.

**Mendelian inheritance:** Mendelian segregation of alleles was confirmed in nine families with a total of 24 offspring-dam pairs.

**Chromosomal location:** Marker *VOLP67* was found to be X-linked, the rest were unassigned autosomal.

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## A single nucleotide (T→G) polymorphism within intron 23 of the canine *BRCA1* gene

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**Source/description:** A forward primer complementary to exon 23 and a reverse primer complementary to exon 24 were designed to amplify intron 23 of the canine *BRCA1* gene<sup>1</sup> (GenBank accession no. U50709). The target region was amplified from DNA samples from six mixed-breed dogs. The amplified fragments were digested with *Cleavase* in a cleavage fragment length polymorphism, CFLP (3rd Wave Technologies) reaction, according to instructions of the manufacturer (Life Technologies). A variant band pattern was observed in one of the six dogs tested when the cleavage reactions were carried out at 45 °C. Direct sequencing of intron 23 from these dogs revealed a T→G transversion near its 5' end (GenBank accession no. AF159258). The G variant destroyed an *AseI* restriction site. Primers complementary to intron 23 were designed to amplify a 217-bp DNA fragment containing this single nucleotide polymorphism (SNP). To serve as a positive control site for restriction, the reverse primer was engineered to incorporate a second *AseI* site with the substitution of T instead of the A at position 22 of the primer, as indicated in bold. Digestion with *AseI* cleaves the PCR product into fragments, 137, 61, and 19 base pairs (allele 1) or 198 and 19 base pairs (allele 2).

#### Primer sequences:

Exon 23 F: 5'-CCAGTGGTAGTCGTGCAGCC-3'

Exon 24 R: 5'-TGCGGGATCAGGTAGGTGTC-3'

Intron 23 SNP F: 5'-CATGGTAAGTCTTGGCTGTAAGC-3'

Intron 23 SNP R: 5'-GGGTGATCTGTGGTGAATTAATC-3'

**PCR conditions:** 25 μl reactions, containing 120 ng of genomic DNA, 0.6 units of *Taq* DNA polymerase (Gibco BRL), and 10 μM of each primer 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, and 20 mM of Tris-HCl pH 8.0 were carried out. Reactions were denatured at 94 °C for 4 min, followed by 35 cycles of 95 °C for 1 min, 62 °C for 1 min and 72 °C for 3 min, and a 10 minute extension at 72 °C. Restriction with *AseI* was carried out by adding 10 units (1 μl) of the enzyme (New England Biolabs) directly to the PCR reaction tube and incubating for 2 h at 37 °C. The resulting fragments were separated on 2% agarose gels and stained with ethidium bromide for genotyping.

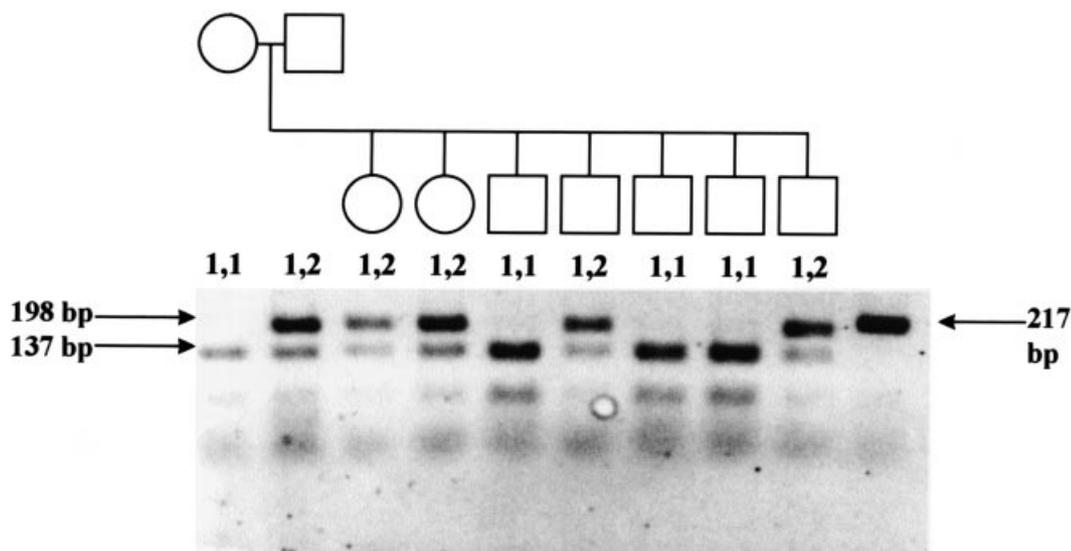


Fig. 1. Reverse image of ethidium bromide stained 2% agarose gel indicating the genotypes in the pedigree shown. Sizes of diagnostic bands are given in base pairs on the left. The last lane contains an undigested control PCR product of 217 bp

**Polymorphism:** In eight unrelated mixed bred dogs, allele 1 showed a frequency of 87.5% and allele 2 a frequency of 12.5%.

**Mendelian inheritance:** Codominant Mendelian segregation pattern was observed in a pedigree of Beagles from the DogMap panel<sup>2</sup> as shown in Fig. 1.

**Chromosomal location:** *Canis familiaris* chromosome 9<sup>3</sup>.

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## Seven novel cosmid-derived canine microsatellites

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**Source/description:** Positive clones were identified in a genomic canine cosmid<sup>1</sup> library with end labeled oligonucleotide probes [(GA)<sub>20</sub> or (CA)<sub>20</sub>]. This cosmid library was established by ligating partially *Sau3A*-digested DNA of a male Bernese Mountain dog into Supercos I (Stratagene, La Jolla, CA). After *Sau3A* subcloning into pUC19 and rescreening, six positive clones *ZuBeCa19* through *ZuBeCa23* and *ZuBeCa25* and *ZuBeCa26* were isolated and sequenced on a LI-COR DNA sequencer model 4000L (LI-COR, Lincoln, NE). The PCR primers shown in Table 1 were designed using the OLIGO 5.0 program (National Biosciences, Plymouth, MN).

**PCR conditions:** Amplifications were carried out in 12 µl containing 2 µl of DNA solution (Chelex 100, BioRad or High Pure PCR Template Preparation Kit, Boehringer Mannheim, Mannheim, Germany), 2.5 pmol of each primer, 0.25 mM of each dNTP, 1 × PCR buffer with 1.5 mM MgCl<sub>2</sub> (Appligene, Gaithersburg, MD) and 0.35 unit *Taq* polymerase (Appligene) in a Perkin Elmer 9600 or 9700 thermocycler. PCR was performed using the following

**Table 2** Summary data for seven canine microsatellites

Locus	Allele size	No. of animals	No. of alleles	Heterozygosity	Amplification wolf	Amplification fox
<i>ZuBeCa19</i>	319–335	51	7	0.51	0.55	+
<i>ZuBeCa20</i>	293–315	52	9	0.75	0.78	+
<i>ZuBeCa21</i>	235–255	49	9	0.73	0.75	+
<i>ZuBeCa22</i>	244–264	51	9	0.56	0.60	+
<i>ZuBeCa23</i>	165–181	52	6	0.59	0.63	–
<i>ZuBeCa25</i>	323–339	52	8	0.73	0.68	+
<i>ZuBeCa26</i>	192–206	52	8	0.82	0.84	+

touch-down program<sup>2</sup>: 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 (Table 1) and 30 s at 72 °C, followed by 16 cycles of 30 s at the lowest annealing temperature. The final extension was for 30 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).

**Polymorphism:** The sizes and number of alleles observed in a panel of 52 dogs, representing 52 different breeds are summarized in Table 2. The calculated heterozygosity and polymorphism information content (PIC)<sup>3</sup> are also given in Table 2.

**Mendelian inheritance:** Codominant inheritance was observed in the DogMap reference family pedigree<sup>4</sup>.

**Other comments:** PCR products could be amplified from wolf and red fox DNA with all primer pairs with the exception of *ZuBeCa23*, which does not amplify from fox DNA (Table 2).

**Acknowledgements:** We thank B. Colomb, E. Garbely, and U. Sattler for their technical assistance and P. Schawalder for providing dog blood samples. This work was supported by the Albert-Heim Stiftung, Switzerland.

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**Table 1** Microsatellite, EBI/GenBank accession number, repeat type, primer sequences and touch-down ranges of markers

Locus	Accession	Repeat type	Primer sequences 5'-3'	Annealing temperature touch-down range (°C)
<i>ZuBeCa19</i>	AJ243475	(GT) <sub>15</sub>	F: CCTAGAGCTGTGGCATTGTTTG R: GAGGGTCATGGGGTAAGAGGA	68–60
<i>ZuBeCa20</i>	AJ243476	(GT) <sub>16</sub> CC(GT) <sub>4</sub> CT(GT) <sub>5</sub> GC(GT) <sub>2</sub>	F: ACCAGCATTTTGAGCCTTTA R: AGCCACCTACGCCTTCTT	54–46
<i>ZuBeCa21</i>	AJ243477	(CA) <sub>18</sub>	F: TGCTGCTGGGGACTGAGA R: CCTCTGAAAAATGTGGGTGATG	68–60
<i>ZuBeCa22</i>	AJ243478	(CT) <sub>10</sub> GT(CT) <sub>4</sub> (CG) <sub>2</sub> (CA) <sub>14</sub>	F: TCCCAGAAGGTGTGTTATGAA R: GCCAAAAGAAAGTCCCTCAGAG	68–60
<i>ZuBeCa23</i>	AJ243479	(TG) <sub>15</sub>	F: CCATCTGTCCCCTCTCTCA R: CGGGCTGCCCTAGACTCC	68
<i>ZuBeCa25</i>	AJ245411	(GT) <sub>18</sub>	F: AGTGTTTGCGGAGGATAGAGC R: CAGAGGAGGGGAAGGACAGT	63–55
<i>ZuBeCa26</i>	AJ245412	(CA) <sub>14</sub>	F: GCTTATAGACATTTGTTGGGAGTG R: CTA CTGAGTGTGAGAGTTGGGGTAT	63–55

## Characterization of ten equine dinucleotide microsatellite loci: *NVHEQ21*, *NVHEQ54*, *NVHEQ67*, *NVHEQ70*, *NVHEQ75*, *NVHEQ77*, *NVHEQ79*, *NVHEQ81*, *NVHEQ82* and *NVHEQ83*

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**Source/description:** Equine genomic DNA was digested with *Sau3AI* and size-selected fragments (300–600 bp) were ligated into the *Bam*H1 site of the BluescriptSK+ plasmid. The library was screened with a synthetic (GT)<sub>10</sub> oligonucleotide end-labelled with [<sup>32</sup>P]ATP. Positive clones were picked and rescreened and double positives were sequenced in both directions with dye terminator or dye primer chemistry on an ABI 310 sequencer. Primer sequences and accession numbers to the EMBL GenBank is given in Table 1.

**PCR conditions:** The forward primers (reverse primer for locus *NVHEQ70*) were end-labelled with [<sup>32</sup>P]ATP. The PCR reaction was performed in a GeneAmp PCR System 9600 (Perkin-Elmer Cetus) in a total volume of 10 µl of the following mixture: 30–50 ng

of genomic DNA, 2 pmol of each primer, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl<sub>2</sub>, 0.2 mM dNTP and 0.5 U of *AmpliTaq*. Conditions for the amplification reactions were as follows: 5 min denaturation at 94 °C, 28 cycles consisting of 94 °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 6% denaturing polyacrylamide gel (7.5 M urea) and visualized by exposure to X-ray film.

**Polymorphism:** Microsatellite polymorphism was measured in a panel of 26–35 unrelated animals containing Norwegian Fjord, Norwegian Trotter, Thoroughbred and Standardbred. Alleles were compared to a M13 sequencing ladder and named according to their size in base pairs, and allele frequencies were estimated by direct count assuming no null alleles. Allele sizes with corresponding frequencies and locus heterozygosity (HTZ), probability of exclusion (PE) and polymorphism information content (PIC) are given in Table 2. Allele sizes for each locus for the four horses from the 1994 ISAG/ISBC Thoroughbred Standardization Test are shown in Table 3.

**Mendelian inheritance:** For all loci except *NVHEQ75*, no deviations from autosomal codominant segregation were observed analysing minimum one half-sib family from the 'International Equine Gene Mapping Workshop'.

**Chromosomal location:** X-chromosome linkage is strongly supported for locus *NVHEQ75* after analysing more than 300 indivi-

**Table 1** Primer sequence, repeat sequence of original clone and accession number to the EMBL GenBank for ten *NVHEQ* microsatellite loci

Locus (Accession no.)	Primer sequence (5'-3')	Repeat sequence
<i>NVHEQ21</i> (AJ245762)	F: CCAGAACCTGGACTGAACAGTGTC R: GAATGTGCTTGATGCAGAAGAAGG	(GT) <sub>12</sub>
<i>NVHEQ54</i> (AJ245763)	F: AGATGTCCACCTTCTCGCTG R: CGGGGCTTTTAGGAGGTAACATA	(TG) <sub>12</sub>
<i>NVHEQ67</i> (AJ245764)	F: GCTCACTCAACTCCCAGAG R: GGATTAGATTACCCAGACAACT	(GT) <sub>6</sub> AT(GT) <sub>3</sub> AT(GT) <sub>5</sub>
<i>NVHEQ70</i> (AJ245765)	F: GCTGGTCAAGTCACACTGTG R: AACCTCACCCCAAGTTGTAT	(CA) <sub>19</sub>
<i>NVHEQ75</i> (AJ245766)	F: ATAACCCTGCTTACCCCTCTGT R: CAGGTGACATATCCCAAGGTGTA	(AC) <sub>12</sub>
<i>NVHEQ77</i> (AJ245767)	F: ATAATCTCACCGTTGCTACTA R: TTAGCTATGCTTCATATCACCC	(GT) <sub>10</sub>
<i>NVHEQ79</i> (AJ245768)	F: ATTGCCTGTGCTGAGATGG R: GCAAATTGCCTCTGTATCACAC	(TG) <sub>13</sub> (AG) <sub>17</sub>
<i>NVHEQ81</i> (AJ245769)	F: TTCTGGATGAAGTGCAGCC R: GGACAGTGGAGGTGGAGGATA	(TG) <sub>9</sub> GT(TG) <sub>2</sub>
<i>NVHEQ82</i> (AJ245770)	F: TGTGGCAGCATCCCACAAC R: CCTCCATTTTGTGCGTTAGCG	(CA) <sub>14</sub>
<i>NVHEQ83</i> (AJ245771)	F: TTGTTGCTGTGCTGGTG R: TGGAATGGAGGATGAACAGATA	(GT) <sub>10</sub>

**Table 2** Allele sizes with corresponding allele frequencies and polymorphism information for ten *NVHEQ* microsatellite loci. M allele for alphabetic nomenclature is shown in bold

<i>NVHEQ</i> locus	21	54	67	70	75	77	79	81	82	83
Allele size/frequency	151/0.31 <b>153</b> /0.40 155/0.27 161/0.02	172/0.23 <b>182</b> /0.65 186/0.12	187/0.35 <b>195</b> /0.65	189/0.06 193/0.21 <b>197</b> /0.27 199/0.02	201/0.13 203/0.31 110/0.06 112/0.27	98/0.08 <b>134</b> /0.91 102/0.40 136/0.09 104/0.09 <b>106</b> /0.04 108/0.06 110/0.06	172/0.47 174/0.29 <b>184</b> /0.15 186/0.04 188/0.03 190/0.02	<b>166</b> /0.98 168/0.02	127/0.22 129/0.10 <b>131</b> /0.30 133/0.28 141/0.10	121/0.10 <b>123</b> /0.90
PE	0.38	0.27	0.18	0.55	0.53	0.07	0.42	0.01	0.54	0.08
PIC	0.60	0.45	0.35	0.73	0.71	0.15	0.61	0.03	0.73	0.16
HTZ	0.68	0.52	0.46	0.78	0.75	0.16	0.68	0.02	0.78	0.18

**Table 3** Allele sizes for the four reference horses of the 1994 ISAG/ISBC Thoroughbred Standardization Test

Locus	Horse no. 1	Horse no. 2	Horse no. 3	Horse no. 4
NVHEQ21	155	155	153/155	153/155
NVHEQ54	182	182	182	172/182
NVHEQ67	187	195	195	195
NVHEQ70	203	193	193/203	193/203
NVHEQ75	112	108/112	112	102/112
NVHEQ77	134	134	134	134
NVHEQ79	172	172/184	172	172
NVHEQ81	166	166	166	166
NVHEQ82	127/131	131	127/131	127/141
NVHEQ83	123	121/123	123	123

duals of known sex observing homozygous males only while both hetero- and homozygous females were observed. Chromosomal locations for the remaining nine loci are unknown.

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## New mutation in exon 2 of the bovine leptin gene

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*Source/description:* PCR fragments from two unrelated Belgian Blue Crossbred animals, using primers described by M. Pfister-Genskow<sup>1</sup>, were cloned in pCR<sup>TM</sup>II cloning vector (TA Cloning Kit, Invitrogen, San Diego, USA) and sequenced using the ALFexpress<sup>TM</sup> Auto-read<sup>TM</sup> Sequencing Kit (Pharmacia, Uppsala, Sweden). The following two mutations were detected: ggCgatctaccAc (r) ggTgatcttaccG. At the protein level these mutations would be found as an Alanine (r) Valine and a Glutamine (r) Arginine substitution. Based on this sequence new primers were constructed for the routine genotyping of the C to T mutation.

*PCR primer sequences:*

5' primer: GGGAAGGGCAGAAAGATAG

3' primer: TGGCAGACTGTT\*GAGGATC

\* incorporated mismatch in view of destroying a native Hph I recognition site (GGTGAN<sub>8</sub>)

*PCR conditions:* PCR amplifications were performed in 10 µl on a T3 Thermocycler (Biometra, Göttingen, Germany). The reaction mixes comprised 20–100 ng genomic DNA, 2 mM MgCl<sub>2</sub>, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 10 pmol of each primer, 200 µM dNTPs and 0.5 U Taq Platinum polymerase (Life technologies, Merelbeke, Belgium). The thermal cycling profile was: 94 °C (10 min) 1 cycle, 94 °C (30 s), 51 °C (30 s), 72 °C (1 min) 30 cycles, 72 °C (10 min) 1 cycle.

*Analysis of PCR-generated fragments:* The amplified fragments were digested overnight with 5 U of HphI (Westburg, Leusden, Netherlands). The digestion fragments were identified on a 2% agarose gel. The length of digestion fragments were 331 bp and 311 bp + 20 bp for the wild-type and mutant-type allele, respectively.

*Allele frequency:* Four hundred and twenty-one unrelated animals were genotyped. The following breeds were included in this study: Belgian Blue, Charolais, Piedmonte, Limousin, Red and Black Holstein, Belgian Blue Crossbred, Red Pied and Red West Flanders, Blonde d'Aquitaine, MRY, Jersey, Main-Anjou. Allele frequencies (for breeds from which at least 18 animals were investigated) are shown in Table 1.

**Table 1.** Allele frequencies of the investigated animals

Breed	Wild type allele freq.	Mutant type allele freq.	N*
Belgian Blue	0.713	0.287	54
Piedmonte	0.736	0.264	36
Blonde d'Aquitaine	0.789	0.211	47
Charolais	0.72	0.28	18
Limousin	0.887	0.113	53
Red Pied	0.616	0.384	56
Belgian Blue Crossbred	0.875	0.125	28
Red West Flanders	0.518	0.482	27
Red Holstein	0.755	0.245	53
Black Holstein	0.704	0.296	49

\*N: number of animals investigated.

*Mendelian inheritance:* Codominant Mendelian segregation for the two alleles was observed in three families.

*Chromosomal location:* The bovine leptin gene has been assigned to chromosome 6q32 by fluorescence *in situ* hybridization.<sup>1</sup>

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*References*

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