

## Detection of an *Mbo*I RFLP at the porcine clotting factor IX locus and verification of sex linkage

E N Signer, J A L Armour, A J Jeffreys

Department of Genetics, University of Leicester, Leicester LE1 7RH, UK

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**Source/description:** The clotting factor IX gene is X-linked in humans. The porcine cDNA sequence was obtained from the EMBL database (Sarkar *et al.* 1990). Human genomic DNA was compared with pig cDNA to identify conserved exon sequences. PCR primers were designed for exon 7 and 8 and used to amplify across intron 7 (668 bp in humans) from pig genomic DNA. The product of two males (Chinese Meishan and Wild Boar) was sequenced into the intron (498 bp, EMBL acc. no. X92427) starting from exon 8. The coding part was confirmed in the pig cDNA and an intronic *Mbo*I site polymorphism detected.

### Primer sequences:

Exon 7 primer: 5'GTAAAATTGATGCATTCTGTGG3'

Exon 8 primer: 5'CGCCTTTGCTCTGTAGGTTTC3'

Product size: approximately 1350 bp.

**PCR and electrophoresis conditions:** 50–100 ng genomic DNA was amplified for 40 cycles (95°C, 1 min; 58°C, 1 min; 70°C, 2 min) in 10 µl reaction buffer (Jeffreys *et al.* 1991) plus 1 µM of each primer and 0.5 U Taq polymerase. After amplification 20 µl *Mbo*I reaction mixture was added (containing 10 U *Mbo*I and 1× reaction buffer) and incubated at 37°C overnight. Subsequent electrophoresis was done either on 2% agarose or 3% Nusieve/1% agarose gels in 1×TBE containing ethidium bromide at 5 V/cm for at least 6 h.

**Polymorphism:** The absence or presence of the *Mbo*I site is represented by a ~360 bp fragment (allele A) and a ~330 bp fragment (allele B) respectively, and is due to a C to T transition at position 72 in intron 7. Three additional invariant bands (370 bp, 290 bp and 250 bp; Fig. 1) were also present plus other non-reproducible fragments presumably arising through ectopic mispriming.

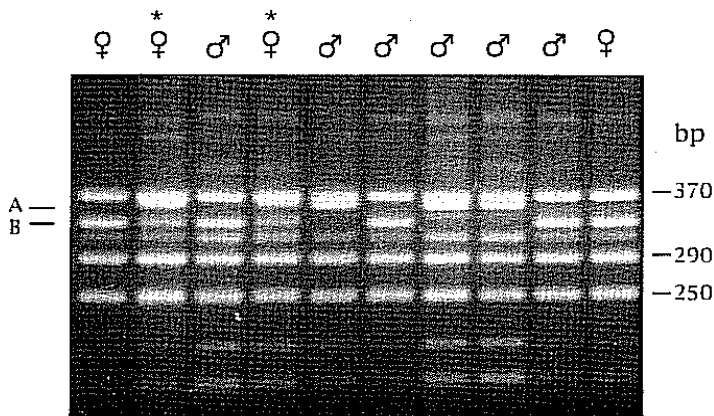


Fig. 1. Genotype of ten pigs at clotting factor IX intron 7 after PCR amplification and digestion with *Mbo*I. Alleles A and B are indicated by arrows. Heterozygous individuals are marked (\*). The sizes of reproducible but invariant fragments are given on the right.

**Allele frequency:** Allele B occurred predominantly in the European pig breeds analysed (British Saddleback, Duroc, Landrace, Large White, Pietrain and Wild Boar). No exceptions were found among 29 unrelated individuals and all females were homozygous at this position. In contrast, this allele was absent in almost all 11 unrelated Chinese Meishan pigs tested which had allele A instead. Exceptions were one male (B) and one female (A/B).

**Mendelian inheritance:** Sex-linked segregation of the alleles was observed in five informative three-generation PiGMap reference families (105 individuals) (Archibald *et al.* 1995). No heterozygotes were seen in 73 males.

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### References

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Correspondence: E N Signer

## Trinucleotide repeat polymorphism at the alpha-enolase/tau-crystallin locus in ducks

J C Cathey, L M Smith, R J Baker, J A DeWoody

Departments of Range and Wildlife Management and Biology, Texas Tech University, Lubbock, TX 79409 USA

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**Source/description:** The DNA sequence for alpha-enolase/tau-crystallin gene in the Peking duck (*Anas platyrhynchos*; GenBank acc. no. M55132) contains a TTA<sub>10</sub> trimeric repeat array (Wistow *et al.* 1988, Kim *et al.* 1991). Polymerase chain reaction primers were designed from the base pair composition within the flanking

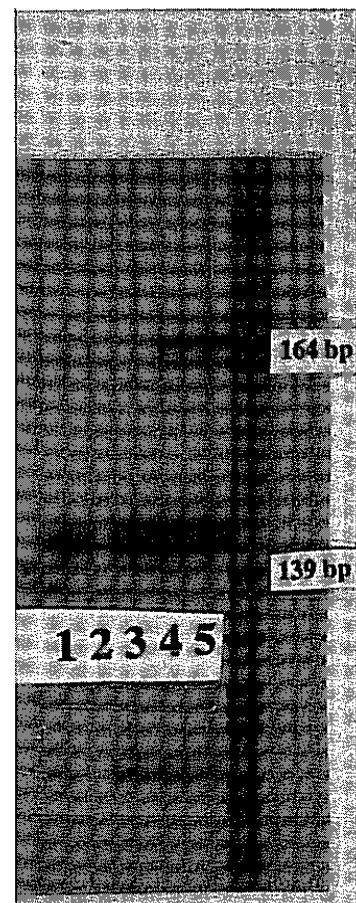


Fig. 1. Mendelian inheritance was established by PCR amplification of this trimeric repeat in a family of wood ducks. PCR products were loaded in the following manner: lane 1 contains the mother, lanes 2–5 include siblings from the same clutch. The G lane of M13 (Sequenase Version 2.0, United States Biochemical) was used as a molecular size standard.

sequences. These primers generate a 160-bp PCR product. Polyacrylamide gel electrophoresis was used to detect two length polymorphisms in a family of wood ducks (*Aix sponsa*) (Fig. 1).

*Primer sequences:* primer 1: 5'GGATTGGAGATTTTCAGGAGC3'  
primer 2: 5'AGGGAAGTGTATGCCCCA3'

*PCR conditions:* The reaction mixture included: primers 1 and 2 each at 20 µM, dNTPs each at 200 µM, 1.0 unit of Taq polymerase (Promega Biotec), 50 mM KCl, 10 mM Tris-HCl, pH 9.0, 1% Triton X-100, and 1.5 mM MgCl<sub>2</sub> for each 25 µl reaction. Amplification of the PCR product was conducted at the following parameters: denaturing at 95°C (60 s); annealing at 55°C (30 s); extension at 72°C (30 s). The reaction was carried out for 35 cycles.

*Polymorphism and mendelian inheritance:* Two alleles were identified in a family of wood ducks (a mother and 4 siblings) who exhibited these alleles in a manner consistent with Mendelian inheritance. Additionally, 3 alleles were detected among 5 mottled

ducks (*Anas fulvigula*). However, no variation was detected among 50 Canada geese (*Branta canadensis*) that were examined.

*Chromosomal location:* Unknown.

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Correspondence: J C Cathey

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