Detection of an MboI 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
Accepted 30 October 1995

Source/description: The clotting factor IX gene is X-linked in humans. The porcine cDNA sequence was obtained from the EMBL database (Sukkar 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 Bear) was sequenced into the intron (496 bp, EMBL acc. no. X92427) starting from exon 8. The coding part was confirmed in the pig cDNA and an intronic MboI site polymorphism detected.

Primer sequences:
Exon 7 primer: 5’GAAAAATTGATGATTCTGTGC3’
Exon 8 primer: 5’CGCCCTTGCCTCTGTAGGC’
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; 72°C, 2 min) in 10 μl reaction buffer [Jeffreys et al. 1989] plus 1 μM of each primer and 0.5 U Taq polymerase. After amplification 20 μl MboI reaction mixture was added (containing 10 U MboI and 1xreaction buffer) and incubated at 37°C overnight. Subsequent electrophoresis was done either on 2% agarose or 3% NuSieve/1% agarose gels in 1xTBE containing ethidium bromide at 5 V/cm for at least 3 h.

Polymorphism: The absence or presence of the MboI 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.

Allele frequency: Allele B occurred predominantly in the European pig breeds analyzed (British Saddleback, Duroc, Landrace, Large White, Pietrain and Wild Bear). 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.

Acknowledgements: This work was supported by the EC BIOTECH program PigMaP, the Wellcome Trust and the Royal Society.

References

Correspondence: E N Signer

Trinucleotide repeat polymorphism at the alphanelosil-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
Accepted 18 September 1995

Source/description: The DNA sequence for alpha-elongase/tau-crystallin gene in the Peking duck (Anas platyrhynchos; GenBank acc. no. M55132) contains a TTA10 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. Genotype of ten pigs at clotting factor IX intron 7 after PCR amplification and digestion with MboI. Alleles A and B are indicated by arrows. Heterozygous individuals are marked [*]. The sizes of reproducible but invariant fragments are given on the right.

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.

© 1996 International Society for Animal Genetics, Animal Genetics 27, 121–131
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'GGATTGGAGATTTCAGGAGC3'
- Primer 2: 5'AGGGAACTGATGCCTCCA3'

**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 MgCl₂ 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.

**Acknowledgements:** The authors thank O.E. Rhodes, E.P. Rent, and R.A. Kennamer for providing DNA samples of the wood duck family. This research was supported by a grant provided by the United States Fish and Wildlife Service and the Central Flyway Waterfowl Council.

**References**


**Correspondence:** J C Cathey