Use Of Hair Root as a Source of DNA For The Detection of Heterozygotes For Recessive Defects in Cattle

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Blood based polymerase chain reaction (PCR) methods are commonly used to genotype cattle for a variety of characteristics. However, collection and transport of blood samples contribute to the total cost of tests, and community concerns over human health hazards associated with blood have complicated the transport of even bovine blood. A further disadvantage of blood-based PCR methods is that hemopoietic chimerism is highly likely to cause erroneous results when genotyping dizygotic twins. For example, genotyping for milk proteins (Leveziel *et al.* 1988), percentage verification using DNA 'finger prints' (Plante *et al.* 1992) and detection of heterozygotes for recessive defects (Healy *et al.* 1994) have been shown to be complicated by chimerism.

Thompson *et al.* (1992) described the use of hair roots and buccal cells to genotype humans for cystic fibrosis. Because such sources of DNA offer a means for avoiding the above disadvantages associated with blood leukocytes, we investigated the use of hair root DNA to genotype cattle for citrullinaemia (Dennis *et al.* 1989), leukocyte adhesion deficiency (Shuster *et al.* 1992), maple syrup urine disease (Healy and Dennis 1994), protoporphyria (G. Johnson, personal communication) and a Msp1 polymorphism associated with generalized glycogenesis in Brahmans (R. Drinkwater, personal communication). For ease of collection, coarse hairs were taken from the switch of the tail. The proximal 5 mm from 12 hairs was dissected and placed in 50 µL of PCR buffer (10 mol/L Tris-HCI, pH 8.3; 50 mol/L KCI; 1.5 mol/L $MgCI_2$) containing 5 µg of proteinase K, and incubated at 60°C for 30 min, followed by 100°C for 10 min (Thomson *et al.* 1992). The extracts were held at 4°C for 2 days before analyses. DNA was isolated from blood using a salting-out procedure (Miller *et al.* 1988). Identical results were obtained with blood and hair root DNA from 2 animals homozygous wild-type (normal) and 2 heterozygous for each of the respective nucleotide various.

Between April 1994 and January 1995 we then analysed hair samples from 70 and 51 Holstein/Friesians for citrullinaemia and bovine leukocyte adhesion deficiency, respectively, 38 Poll Hereford for maple syrup urine disease, 151 Limousins for protoporphyria, and 18 Brahmans for the Msp1 polymorphism. Twenty-eight of the samples were collected from cattle at this institute. The other 300 samples were sent to the laboratory via the postal system. Upon arrival at the laboratory, hairs were held at either 4°C for up to 2 weeks or at -20°C for up to 12 months before digestion with proteinase K. Digests were held at 4°C for up to 4 weeks or at -20°C for 6 months before PCR analysis.

Unequivocal results were obtained with 324 (98.7%) of the 328 samples. With 2 samples, taken 1 month apart from a Holstein-Friesian bull calf, bovine leukocyte adhesion deficiency sequence was not amplified from the digests of the hair roots. With blood as the source of DNA, we established that this animal was homozygous wild-type (normal) for this defect. Sixteen of the 18 samples submitted from Brahmans consisted of only 2 hairs. These samples were digested in 20 to 50 µL of proteinase K. No amplification was obtained with 2 (12.5%) of these samples. All hairs do not grow continuously. A phase of active growth (anagen) is followed by a resting period (telogen). To the naked eye it may be difficult to distinguish between the actively growing follicle and the 'club' of the telogenic phase that lacks nucleated cells. Our success with amplification from 99.4% of 312 samples when we processed 12 roots suggests that in the tail switch some hair growth persists throughout the year. In preliminary experiments, we amplified citrullinaemia, bovine leukocyte adhesion deficiency and maple syrup urine disease sequences from 10 µL digests of 1 hair root. This suggests that failure to amplify the Msp 1 sequence from 2 Brahmans, when only 2 hairs were submitted, reflects the proportion of switch hairs in telogen, not necessarily a lack of target DNA.

Coarse hairs from the switch of the tail are easy to collect from adult cattle in handling yards or dairy parlors, with minimal risk to the operator. Using artery

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forceps to grasp the fine hairs from the tip of the tail, suitable samples can be taken from very young calves, even those wet with fetal fluids. For routine testing we suggest 20 hairs should be taken from the switch of the tail, avoiding faecal contamination. The entire hairs, or 5 cm of the proximal end, should be placed in a small plastic bag or envelope, labelled with the identity of the animal and, without delay, posted to the laboratory.

The bovine α and β mannosidase genes have now been cloned (Tollersrud, personal communication and Friderici, personal communication, respectively). Definition of the mutations responsible for α and β mannosidosis will allow hair roots to be used in the detection of heterozygotes for these lethal defects. It is our policy to report results of analyses for samples, not for animals if we have not personally identified the subject and collected the sample. If subsequently required, the validation of sample and animal identity is possible, as DNA suitable for 'fingerprinting' can be amplified from hair roots (Ron *et al.* 1994). Furthermore, we have shown that hair roots, and their digests, remain a suitable target for PCR amplification for many months, if not years, when stored at -20°C.

References

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Abstract

Types of vaccines and principles of protection

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Several types of vaccines are currently used or being used or being developed. They can be split into two categories: live and inactivated vaccines. Traditional live vaccines are attenuated by various methods, including growth in usual conditions (for bacteria) or in cells or animal species to which they are not initially adapted (for viruses). New generation of live vaccines can be generated either by directed mutagenesis of virulence genes or by cloning genes of immunogenic proteins into vectors i.e. other bacteria or viruses with attractive properties of safety and efficiency. Conventional inactivated vaccines are made by the use of physical or chemical treatment of microrganisms. As the immunogenic components of microorganisms are more and more well identified, they can be used to make subunit vaccines, in which the antigenic fraction is derived by purification of antigens, or by *in vitro* gene expression of proteins or chemical synthesis of peptides. Finally, recent advances demonstrated that direct inoculation of an immunogenic protein encoding gene into muscle (known as genetic immunization) was able to elicit antibody and cellular immune responses. Systemic and mucosal immunity conferred by live and inactivated vaccines are different. Shortly, in contrast to live vaccines, inactivated vaccines are unable to elicit a strong mucosal immunity and cytotoxic T-cell response. Nevertheless use of adjuvants improves the potency of inactivated and even some live vaccines. Besides conventional adjuvants, others are currently being developed, both for improving systemic and mucosal immunity: microparticles, liposomes, immunostimulating complexes, cytokines and bacterial enterotoxins like the cholera toxin.

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