PCR-RFLP based genotyping of cattle using DNA extracted from hair samples

Pushpendra Kumar*, V Choudhary, T K Bhattacharya, B Bhushan and Arjava Sharma
Molecular Genetics Laboratory, Animal Genetics Division, Indian Veterinary Research Institute, Izatnagar 243 122, India

Received 4 July 2003; revised 22 March 2004; accepted 10 April 2004

A simple method of genotyping of farm animals using DNA extracted from hair samples is described. Hair samples of 35 F × H (Holstein Friesian × Hariana) crossbred cattle were processed for isolation of genomic DNA. These DNA samples were used for genotyping of insulin-like growth factor binding protein 3 (IGFBP 3) gene using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Thus, hair samples could be an easy and reliable source of DNA for genotyping of farm animals or any other molecular genetic research work where DNA is needed as an experimental material for the study.

Keywords: DNA, genotyping, hair, PCR, FLP
IPC Code: Int.Cl.7 C12N15/06, 15/10

Blood based polymerase chain reaction (PCR) methods are commonly used to genotype domestic animals for variety of characteristics. However, collection and transportation of blood samples contribute to the total cost of the tests and community concerns over human health hazards associated with the blood. Hair samples seem to be an easy source of genomic DNA isolation for subsequent genetic analysis1. Most of the DNA in hair is located in the root and surrounding sheath cell2. As measured by the fluorescence of DNA dye complexes in the crude lysates of the hairs, the root end of the freshly plucked hairs may contain as much as 0.5 µg DNA, whereas hair shafts contain too little DNA2. Attempts have been made to isolate DNA from hair samples but these methods are either time consuming or require expensive chemicals. In this paper, the authors report a rapid and simple procedure for isolation of genomic DNA from hair roots for genotyping of farm animals or for any other molecular analysis where DNA is used as a basic experimental material.

Hair samples were collected from 35 F × H (Holstein Friesian × Hariana) crossbred cattle from LPM section, IVRI, Izatnagar. Approximately 10 plucked hairs from each animal were rinsed in deionized water, followed by rinsing in 100% ethanol. The hairs were dried for about 10 min and hair bulb from each shaft was removed (not longer than 5 mm) with an ethanol/heat sterilized scalpel. The hair bulbs from each animal were then transferred in a 1.5 ml micro-centrifuge tube. Scissors were wiped between samples with tissue paper and then 70% ethanol to avoid cross contamination between the hair samples2. The micro-centrifuge tube was spun at 13,000 rpm in a centrifuge for few sec to collect follicles at bottom of the tube. A 50 µl extraction buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl; 1.5 mM MgCl2), containing 5 µg of proteinase K, was added in this tube and incubated at 60°C for 30 min, followed by 95°C for 10 min. Finally, tubes were spun at 13,000 rpm. The extracted DNA was stored at –20°C for further use. The hair samples may be kept at –20°C for years before digestion with proteinase K.

Concentration of the DNA was estimated spectrophotometrically at 260 nm using 100 µl of 1:100 dilutions of DNA. Subsequently, quality of the DNA was checked by 1% agarose gel electrophoresis3. The primers 1 and 2 (forward and reverse) were designed on the basis of the published bovine nucleotide sequence of cDNA of IGFBP 34,5 (Gene Bank Acc No. 83465) and have the following primer sequence:

Primer 1: 5’- CCAAGCGTGAGACAGAATCA- 3’
Primer 2: 5’- AGGAGGGATAGGAGCAAGTT -3’

The PCR was performed in 25 µl reaction volume containing 1 X PCR buffer [50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1% Triton X 100], 1.5 mM MgCl2, 200 µM of each dNTPs, 0.4 µM of each primer, 50-100 ng genomic DNA and 0.4 U Taq polymerase. The amplification was carried out using a pre-programmed thermal cycler (PTC-200 DNA engine, MJ Research) with the following PCR conditions: initial denaturation for 1.5 min at 97°C, followed by 35 cycles of 1 min each for denaturation at 94°C, annealing at 60°C and extension at 72°C and final extension of 5 min at 72°C.

*Author for correspondence:
Tel: 91-581-2303382, Fax: 91-581-2303284
E-mail: pushpendra@ivri.up.nic.in
Amplified product (5 μl) was checked in 1.5% agarose gel electrophoresis for confirmation of the amplicons. About 12.5 μl PCR product was digested with 5 U Hae III restriction enzyme (tetracutter with recognition sequence as GGCC) at 37°C for 2 hrs. Restriction digested PCR products were electrophoresed in 4% agarose gel in TAE buffer.

The yield of genomic DNA varied from 0.5-2.0 μg, which is a sufficient amount for 10-25 PCR and subsequent genotyping. The yield of genomic DNA from hair samples depends on the number of hairs used and the quality of hairs. Generally hair root region provides quite a good amount of DNA. Agarose gel electrophoresis examination of the genomic DNA revealed intact single band indicating no shearing of the DNA; however, intensity of band was less as compared to that isolated from blood (Fig. 1).

A 651 bp fragment of IGFBP 3 gene was amplified using primers 1 and 2 (Fig. 2). The amplified fragment of same size was also reported in indicine cattle. The digestion of PCR product with Hae III restriction enzyme revealed three restriction fragment patterns of sizes, i.e. 199, 164, 154, 56, 36, 18, 16 and 8 bp (AA genotype); 215,164, 154, 56, 36, 18 and 8 bp (BB genotype); and 215, 199,164, 154, 56, 36, 18, 16 and 8 bp (AB genotype), and thus was found to be polymorphic (Fig. 3). The above results clearly show the suitability of the genomic DNA, isolated from the hair samples, for PCR amplification and enzymatic reactions. All three genotypes have also been reported in various breeds of taurine cattle (Holstein, Angus and Hereford). However, only AA genotype has been found in indicine cattle, sheep and Buffalo.
Peripheral blood is the usual source of genomic DNA isolation and the classical genomic DNA isolation procedure includes proteinase K digestion and phenol:chloroform extraction to remove different cellular proteins. In the present study, however, hair samples were used as a source of genomic DNA because hair is the easily available material for the isolation of DNA and does not require complicated procedures. The method described for genomic DNA isolation from hair roots resulted in good quality of DNA in all the samples and IGFBP 3 gene fragment was amplified using these DNA samples as template. Further, the methodology adopted in this study is rapid and easy. Though, the amount of DNA isolated varied from sample to sample but the quantity was sufficient enough for a number of PCR reactions needed to perform genotyping experiments. Thus, it can be concluded that the genomic DNA isolation from the hair cells can be used for PCR and enzymatic reactions either for genotyping or any other molecular genetic analysis.

Acknowledgement

The Authors are thankful to the Director, Indian Veterinary Research Institute, Izatnagar, Bareilly for providing necessary facilities to carry out this work.

References