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GENOMIC DNA EXTRACTION PROTOCOLS FROM OVINE HAIR

PROTOCOLOS PARA EXTRAÇÃO DE DNA GENÔMICO DE PÊLO DE OVINOS

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Genomic DNA extracted from animal cells can be used for several purposes, for example, to know genetic variability and genetic relationships between individuals, breeds and/or species, paternity tests, to describe the genetic profile for registration of the animal at association of breeders, detect genetic polymorphisms (SNP) related to characteristics of commercial interest, disease diagnose, assess resistance or susceptibility to pathogens, etc. For such evaluations, in general, DNA is amplified by PCR (polymerase chain reaction), and then subjected to various techniques as RFLP (restriction fragments length polymorphism), SSCP (single strand conformation polymorphism), and sequencing. The DNA may be obtained from blood, buccal swabs, meat, cartilage or hair bulb. Among all, the last biological material has been preferred by farmers for its ease acquisition. Several methods for extracting DNA from hair bulb were reported without any consensus for its implementation. This study aimed to optimize a protocol for efficient DNA extraction for use in PCR-RFLP analysis of the Prion gene. For this study, were collected hair samples containing hair bulb from 131 Santa Inês sheep belonging to the Institute of Zootechny, Nova Odessa - SP. Two DNA extraction protocols were evaluated. The first, called phenol-chloroform-isoamyl alcohol (PCIA) has long been used by Animal Genetic Laboratories, whose procedures are described below: in each microtube (1.5 mL) containing 500 μL of TE-Tween solution (Tris-HCl 50 mM, EDTA 1 mM and 0.5% Tween 20) were added to approximately 30 hair bulb per animal which was incubated at 65°C with shaking at 170 rpm for 2 hours. Then was added 15 μL of proteinase K [10 mg mL^{-1}] and incubated at 55°C at 170 rpm for 6-12 hours. At the end of digestion was added 1 volume of solution phenol-chloroform-isoamyl alcohol (25:24:1) followed by vigorous shaking for 10 seconds and centrifuged at 8000 rpm and 4°C for 10 minutes. The upper phase was transferred to another microtube, and the DNA was precipitated in the presence of 0.3 M sodium acetate (1/10 volume) and 1 mL ethanol at -20°C for 6 hours. Then the samples were centrifuged at 12,000 rpm, 4°C for 30 minutes. The precipitate after drying was resuspended in 50 μL of ultrapure water. The second protocol was standardized by the Laboratory of Biochemistry and Genetics, FMRP - USP, whose procedures are described below: from each animal was used about 3-4 hairs, containing the hair bulb. This sample was transferred to a microtube containing 100 mL of buffer (20 mM Tris HCl, 50 mM KCl, 2.5 mM $MgCl_2$ and 0.5% Tween 20) and 1 μL of proteinase K [10 mg mL^{-1}], and then incubated at 60°C with shaking (160 rpm) for 45 minutes. For denaturation of the proteinase-K, the microtubes were incubated at 95°C for 10 minutes. After extraction by both methods, the DNA was quantified and subjected to PCR using specific primers for Prion gene amplification, associated to the Scrapie. The gel electrophoresis in agarose 1% revealed that the DNA obtained by the two protocols enabled amplification of the fragment of 198 pb, as expected. The amplification product was digested by the restriction enzyme *Ava*II at 37°C for 3 hours, and subjected to electrophoresis on polyacrylamide 10% gel (49:1) resulted in two fragments of 106 and 92 bp, as expected. Based on these results we conclude that both extraction methods allow obtaining DNA samples in quantity and quality satisfactory for PCR-RFLP analysis of Scrapie gene, although the latter was more efficient and economical, non-toxic and use smaller amounts of sample per animal.

Keywords: DNA extraction, ovines, PCR-RFLP.

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