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SHORT COMMUNICATION/NOTA PRÉVIA

Bovine carcass sexing by PCR method

Sexagem de carcaça bovina pelo método de PCR

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SUMMARY

The objective of the present study was to develop a methodology that would permit sexing bovine meat ready for commercialization. A male-specific primer sequence was used, followed by analysis of the amplified product. The method proved to be efficient for sex verification and is of practical utility in the prevention of fraud in beef sale.

KEY-WORDS: Bovine. Carcass. Sexing. PCR.

Beef from male animals is commercially more valuable than beef from female animals, because females are used for reproduction and are normally slaughtered only when older, when the length and rigidity of muscle fibers is increased and the level of collagen is higher resulting in tougher meat of lower quality and thus lower commercial value. Many meat packing plants sell cow's meat as male meat, causing damage to various meat producing sectors and to consumers. The objective of the study reported in this paper was to develop a technique that would permit the sexing of boned, packaged and chilled, ready for sale beef. The principle of the technique is the same as used for sexing preimplanted cattle embryos, and depends on the fact that males and females can be differentiated by polymerase chain reaction (PCR) amplification of a male-specific region of chromosome Y³ using a sexually neutral region as an internal control⁴. The same principle is also useful for the identification of freemartin animals which have an anomaly of the female genital system and which is often observed in animals from births with heterosexual twins, especially when the frequency of XY cells is low².

The experiment was conducted at BIOGEM (Laboratory of Biotechnology and Molecular Genetics) of the Department of Genetics, Institute of Biosciences, São Paulo State University (UNESP), Botucatu campus. Boned, packaged and chilled meat samples (36) were collected from butcher shops in different regions of the State of São Paulo with the help of representatives of the butchers' union. The amplified male-specific DNA region was the BRY1 region¹ of 307 bp while the sexually neutral region was a portion of the GH gene⁶ consisting of 223 bp.

Genomic DNA was extracted by the non-phenolic method using digestion with proteinase K and precipitation with NaCl and alcohol⁵. At the end of the DNA extraction procedure DNA was quantified in a spectrophotometer at 260 and 280 nm and its integrity was determined on 0.8% agarose minigel. The DNA samples were stored at 4°C in 1.5 ml Eppendorf tubes.

DNA regions of interest were PCR amplified in a final volume of 25 µl containing 50 ng of genomic DNA, 0.2 mM of each primer, 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 2.0 mM MgCl₂, 0.2 mM of each dNTP and 1.25 U Taq-DNA-polymerase. One drop of mineral oil was added to each reaction mixture to prevent evaporation.

The reactions were carried out in an thermocycler (M.J. Research) according to the following steps: initial denaturation at 95°C for 3 minutes followed by 32 cycles as denaturation at 95°C for 1 minute, annealing at 58°C for 30 seconds, extension at 72°C for 1 minute, and a final extension at 72°C for 3 minutes. After the last cycle was completed in step 4, the temperature was reduced and kept at 4°C for cooling.

Electrophoresis was carried out using 2% ethidium bromide stained agarose gel, at 120V for 1 hours. After electrophoresis, the amplified fragments were visualized under U. V. light (Fig. 1).

We analyzed 36 samples identified as belonging to males, 17 of which (47.2%) presented a discordant result, i.e., they actually belonged to females. This method was a cheap and efficient technique for the determination of cattle sex from the carcass. It is technically simple and rapid, permitting its utilization in the prevention of fraud in the commercialization of beef.

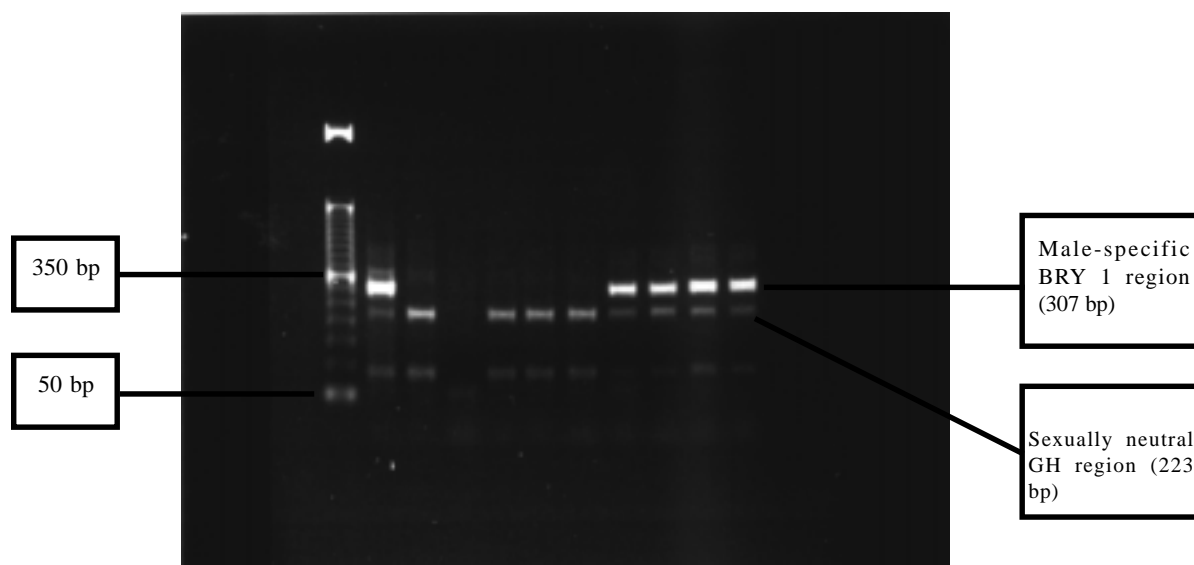


Figure 1

Ethidium bromide stained agarose gel electrophoresis of bovine DNA. Lane 1, DNA ladder 50 bp. Lane 2, amplification of DNA from a bull's blood sample used as control. Lane 3, amplification of DNA from the blood sample of a cow used as control. Lane 4, no DNA. Lanes 5, 6, 7, 8, 9, 10 and 11, amplification of DNA from meats assumed to have been erroneously sexed. The numbers on the sides of the figure indicate DNA fragments size in base pairs (bp).

RESUMO

Objetivou-se, nesta pesquisa, o desenvolvimento de uma metodologia que permitisse a sexagem de carne bovina pronta para comercialização. Para tanto, utilizou-se *primers* seqüência macho-específica e posterior análise do produto amplificado. O método proposto mostrou-se eficiente para verificar o sexo, bem como sua utilização prática, a fim de evitar fraudes na comercialização de carne bovina.

PALAVRAS-CHAVE: Bovino. Carcaça. Sexagem. PCR.

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