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A PCR assay for sex determination of yak (*Bos grunniens*) meat by amplification of the male-specific *SRY* gene

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ABSTRACT

Yak meat is of great economic importance to the people living in the cold high altitude area. In the present work, a multiplex PCR-based method was proposed for the sexing of yak meat by amplifying the target sequence of male-specific *SRY* gene. In a single reaction set, two DNA fragments of 121- and 290-bp were amplified from male meat DNA using two pairs of primers, but only one DNA fragment of 290-bp was amplified from female meat DNA. The assay proposed herein was applied to raw and heat-treated yak meat samples, and the identification results obtained were in perfect agreement with the anatomical sex of the yak meat samples. The method is fast, reliable and cheap. It is potentially suitable for the sexing of yak meat in routine analyses. It can be considered a helpful tool in the quality control of yak meat and meat products.

1. Introduction

Yak, a herbivore, has been regarded as one of the most remarkable livestock. It lives in and around the Himalayas and further north at altitudes ranging from 2500 to 5500 m with no frost-free, where few other domestic animals can survive (Wiener, Han, & Long, 2003; Zi, Lu, Yin, & Chen, 2008). Yak is reared for multi-purpose use, including meat, milk, wool, fur and other products (Sasaki, 1994). Among the products from yak, meat is of great economic importance to the herdsmen living in the cold high altitude area. Among local people, moreover, yak meat has a flavour akin to game (Wiener et al., 2003) based on the fact that yak meat not only has a fine texture, high protein with low fat content (He & Feng, 2004), but also lacks of anabolic steroids or other drugs (Yin et al., 2009). Therefore, there has been an increasing focus on yak meat and meat products in recent years. On the other hand, however, the quality of yak meat and meat products also has become a matter of great concern for consumers. For the purpose of ensuring the quality of yak meat and meat products, several analytical methods have been developed for the specific differentiation of meat from yak (*Bos grunniens*), cattle (*Bos taurus*), and buffalo (*Bubalus*) (Chen, Bai, Zhou, Zhang, & Wu, 2008; Yin et al., 2009). However, the methods reported do not tell whether the meat is from a male or female.

Sex determination of domestic animal meat has received great attention in recent years. An outstanding example exists in beef, where male beef is designated to be of higher quality than cow or heifer meat, and therefore yields higher prices (Zeleny & Schimmel, 2002). To avoid unfair competition and to assure consumers of accurate labeling, it is necessary to develop reliable methods for determining the gender of the meats.

To date, a range of different methodologies have been developed for determining the gender of meat, mainly based on detecting either hormone or DNA (Zeleny & Schimmel, 2002). In general terms, hormone-based methods include immunochemical determination using ELISA (Simontacchi, Marinelli, Gabai, Bono, & Angeletti, 1999), and chromatographic techniques with mass-spectrometric detection, such as HPLC–MS/MS (Draisci, Palleschi, Ferretti, Lucentini, & Cammarata, 2000) and GC–MS (Hartwig, Hartmann, & Steinhart, 1995, 1997). Most of the methods proved to be highly specific and sensitive, but were not performed on a regular basis for meat sexing due to the technical limitations or the expensive equipment required.

Over the last few decades, DNA-based techniques, especially polymerase chain reaction (PCR)-based methods for mammal sexing have received particular attention, and have proved to be reliable, sensitive, and fast (Appa Rao, Kesava Rao, Kowale, & Totey, 1995; Tagliavini, Bolchi, Bracchi, & Ottonello, 1993). In general,

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these methodologies were developed mainly based on the detection of the X- and Y-chromosome homologous amelogenin genes (*AMELX* and *AMELY*) (Checa, Dunner, & Canon, 2002; Zeleny, Bernreuther, Schimmel, & Pauwels, 2002), and zinc finger genes (*ZFX* and *ZFY*) (Kirkpatrick & Monson, 1993; Zinovieva, Palma, Müller, & Brem, 1995). Alternatively, the bovine-specific repetitive sequence BRY-1 (Matthews & Reed, 1992) and the single copy sequence BOV97 M (Miller & Koopman, 1990) are also used in the analysis for the sexing of bovine. More recently, based on real time PCR, both SYBR Green (Ballin & Madsen, 2007) and TaqMan (Parati, Bongioni, Aleandri, & Galli, 2006) technology for bovine sex determination have been reported. Moreover, studies have demonstrated that *SRY* gene can also be used as a target DNA for embryos sexing by PCR amplification (Fu et al., 2007; Shi, Yue, Ren, Lei, & Zhao, 2008). The purpose of the present work was to develop a fast, reliable, and straightforward protocol for the sexing of yak meat based on the male-specific *SRY* gene using PCR technique.

2. Materials and methods

2.1. Sample selection and DNA extraction

A total of 35 blood samples were randomly collected from the following four Chinese yak breeds: Jiulong (three males, six females), Maiwa (four males, seven females), Datong (three males, four females), and Tianzhu white (three males, five females). Also, 10 raw meat samples (four males, six females) were collected from a local slaughter-house. The collected samples of blood and raw meat were transported to the laboratory under refrigeration, and were stored frozen at −20 °C prior to analysis. In addition to raw meat, 10 pasteurized (72 °C, 30 min) and 10 sterilized (121 °C, 20 min) meat samples also were prepared for analysis in the present work (Fajardo et al., 2007).

We extracted the genomic DNA from blood samples using Blood Genome DNA Extraction Kit (TaKaRa, Dalian, China) and from raw and heat-treated meat samples using the TIANamp Genomic DNA Kit (TIANGEN, Beijing, China). All the procedures were in accordance with the respective manufacturer's instruction. Subsequently, the concentration of DNA extracted from each sample was determined by a spectrophotometer at 260 nm.

2.2. Design of PCR primers

Based on the information obtained from the alignment of the entire encoding region of yak, bovine, sheep and goat *SRY* gene sequences (GenBank Accession Nos. AB077320, Z30327, Z30265 and Z30646, respectively), we designed a pair of primers *YA/SRY-FW* and *YA/SRY-RV* targeting the high-motility-group (HMG) box of *SRY* gene with 100% homology among the species (Fig. 1). This set of primers were potentially suitable for the amplification of a male-specific DNA fragment of 121-bp in length, providing the unambiguous determination of male and female yak. Also, another pair of primers *YA/CA-FW* and *YA/CA-RV* (designed in our previous study, Yin et al., 2009) targeting the 12S rRNA gene of mitochondrial DNA of yak was used in the present work. The amplicon was expected to yield a 290-bp fragment in both male and female yaks, as a positive amplification control. Information on the four primers (*YA/SRY-FW*, *YA/SRY-RV*, *YA/CA-FW*, and *YA/CA-RV*) used in this study is shown in Table 1. The PCR primers were synthesized and PAGE purified by TaKaRa Biotechnology Co., Ltd. (Dalian, China).

2.3. PCR amplification

The PCR reaction was carried out in a 25-μL final volume containing 1 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, Branchburg, NJ, USA), 1 × PCR buffer (TaKaRa, Dalian, China), 1.5 mM $MgCl_2$, 200 μM of each dNTP, 10 pmol of each primer, and approximately 100 ng of genomic DNA. The amplification was carried out in a TC-512 Gradient Thermal Cycler (Techne,

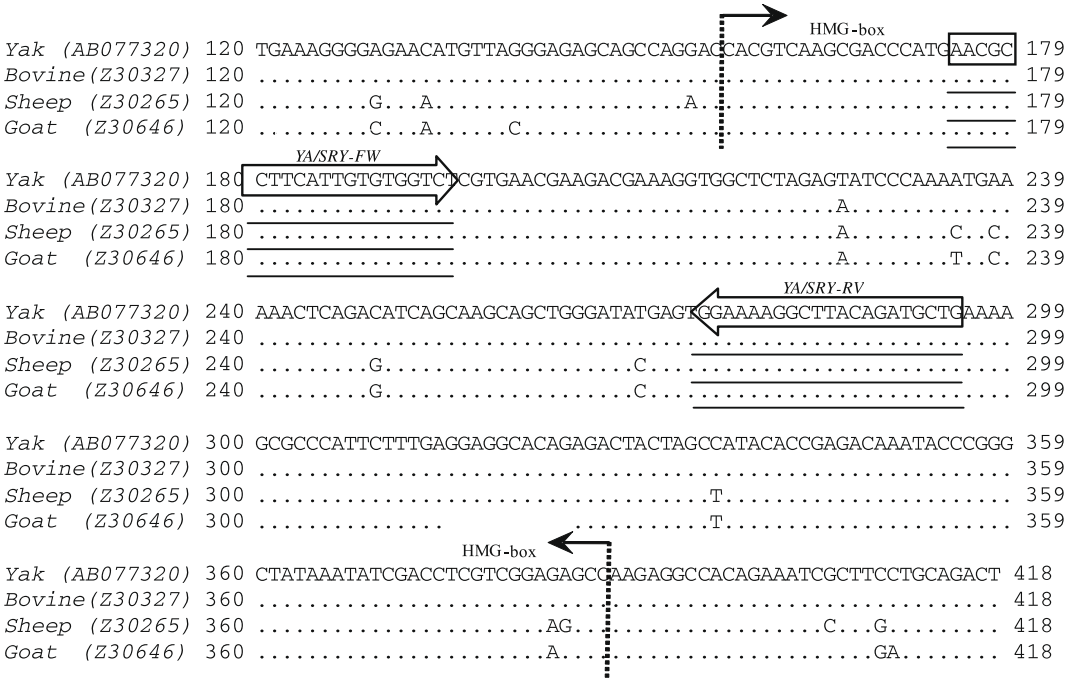

Fig. 1. Partial DNA sequences alignment of the encoding region of yak (*Bos grunniens*, AB077320), bovine (*Bos taurus*, Z30327), sheep (*Ovis aries*, Z30265) and goat (*Capra hircus*, Z30645) *SRY* gene harboring the complete HMG box (shown by horizontal arrows with vertical broken line). The empty arrows indicate the two primers *YA/SRY-FW* and *YA/SRY-RV*, and the positions of the primers are underlined in the sequences of bovine, sheep and goat. The nucleotide positions indicated on both sides of the sequences are compared with the yak sequence (GenBank accession number AB077320). The dot (·) indicates positions with no nucleotide exchanges.