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Short Communication

Use of the *TSPY* gene for sexing cattle

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Abstract

The *Y-encoded, testis-specific protein* (*TSPY*) is a Y-specific gene. The copy numbers of *TSPY* range from 20 to 60 in men and up to 200 in bulls. In this study, we examined the possibility of using the *TSPY* gene to sex cattle. DNA from blood samples of 100 Nelore cattle (50 males and 50 females) from the Nelore Cattle Breeding Program (PMGRN) was screened for *TSPY* by PCR using *TSPY*-specific primers. The assay was highly specific since all male samples were *TSPY*-positive and all female samples were negative. Positive results were also obtained at low DNA concentrations (less than 1 ρg/μL). These results showed that *TSPY* was a good male-specific marker, the usefulness of which was enhanced by the high copy number of the gene. This is the first report to demonstrate the applicability of *TSPY* for sexing cattle.

Key words: bovine, cattle, PCR, sexing, *TSPY*.

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Determining the sex of beef by reliable molecular methods is an important measure to ensure the correct allocation of export refunds, which are considerably higher for male beef (Zeleny *et al.*, 2002). However, although a number of assays have been used to determine the sex of bovine embryos, they are not widely used because of their lack of accuracy and speed (Bredbacka *et al.*, 1995).

A more practical and efficient approach for determining the sex of cattle is to amplify sequences of the Y chromosome by the polymerase chain reaction (PCR) using sequence–specific primers (Alves *et al.*, 2003). The advent of PCR has increased the interest in sexing cattle, and the potential usefulness of this technique has been demonstrated (Bredbacka *et al.*, 1995). Using this approach, male and female beef has been identified by amplifying the *SRY*, amelogenin and *ZFY* genes, as well as other Y-specific sequences (Levinson *et al.*, 1992; Chong *et al.*, 1993; Cui *et al.*, 1994). Homologous but non-identical copies of the amelogenin and *ZFY* genes are also located on the X chromosome and can serve as internal controls for successful amplification using the same sets of primers (Pierce *et al.*, 2000).

The *Y-encoded, testis-specific protein TSPY* (Arnemann *et al.*, 1991) is a Y-specific gene (Affara *et al.*, 1996). *TSPY* homologs exist in several mammalian species, including humans, horses and cattle (Jakubiczka *et al.*, 1993; Schempp *et al.*, 1995; Vogel *et al.*, 1997). The *TSPY* genes are arranged in clusters on the Y chromosome of many mammalian species and form part of a superfamily, TTSN (*TSPY-TSPYL-SET-NAP1L1* genes), with highly conserved autosomal representatives (Vogel and Schmidtke, 1998). In humans and cattle, *TSPY* expression is apparently restricted to male germ cells and their precursors, and begins during fetal development. The cellular site of expression suggests a function in spermatogonial proliferation (Vogel *et al.*, 1997).

In this study, DNA from blood samples of male (n = 50) and female (n = 50) cattle was screened for the presence of *TSPY* using PCR. All of the animals used belonged to the Nelore Cattle Breeding Program (PMGRN). Genomic DNA was extracted from peripheral blood leucocytes using standard methods (Olerup and Zetterquist, 1992), and the sex was confirmed by PCR. The *TSPY*-specific primer sequences were: 5' CCCGCACCTTCCAAGTTGTG 3' and 5' AACCTCCACCTCCTCCACGATG 3'. These primers amplified a 260-bp segment of *TSPY* and were based on sequences reported by Jakubiczka *et al.* (1993) (GenBank accession X74028 - *B. taurus TSPY* gene). The DNA sequence was amplified by an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 64 °C for 45 s and synthesis at 72 °C for 45 s. An additional extension time of 10 min was

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included at the end of the final cycle. The PCR was done using a TGradient thermocycler (Whatman-Biometra). The reaction final volume of 25 µL contained 100 ng of DNA, 10 mM Tris-HCl (pH 8.4), 25 mM KCl, 1.5 mM $MgCl_2$, 100 µM of each dNTP, 1.0 U *Taq* polymerase (GibcoBRL), and 0.05 mM of Y-specific primers. The amplification products were analyzed by electrophoresis in 2% agarose gels stained with ethidium bromide. The sensitivity of the PCR was investigated using different concentrations of male DNA, with the desired amount of DNA contained in 1 µL of each dilution (Table 1).

The PCR using *TSPY*-specific primers was specific since all of the male samples were *TSPY*-positive and all of the female samples were negative. All of the DNA dilutions prepared from male samples gave a positive result (Figure 1).

Table 1 - DNA dilutions and concentrations used to determine the sensitivity of the PCR.

Dilution	Initial DNA concentration	DNA concentration in PCR mix (25 µL)
1	0.1 µg/µL - 100 νg/ µL	4 ηg/µL
2	10 ηg/µL	400 ρg/µL
3	1 ηg/µL	40 ρg/µL
4	100 ρg/µL	4 ρg/µL
5	10 ρg/µL	0.4 ρg/µL
6	1 ρg/µL	0.04 ρg/µL

PCR is a rapid, easy procedure for large scale sexing, and primers derived from many Y-specific sequences have been used to screen blood, meat and blastomere samples (Alves *et al.*, 2003; Zeleny *et al.*, 2002). Manz *et al.* (1998) demonstrated the potential usefulness of *TSPY* for sex diagnosis in equine preimplantation, and Pierce *et al.* (2000) described a highly accurate method for determining the sex of human embryos by real-time PCR of this same gene.

Bovine *TSPY* consists of seven exons that are separated by six introns. The genomic organization of the bovine and human genes is highly conserved and both are part of a Y-specific gene family (Vogel *et al.*, 1997). The copy numbers range from 20 to 60 in men and up to 200 in bulls (Manz *et al.*, 1998).

Park *et al.* (2001) optimized consecutive and multiplex PCR using whole blood from males and different DNA concentrations (minimum = 5 ρg). These authors subsequently successfully applied the method to groups of 1, 2, 4 and 8 blastomeres dissociated from bovine embryos. The sexing efficiency was 92.1, 94.3, 96.3 and 100%, respectively.

In conclusion, primers derived from a *TSPY* sequence allowed the accurate detection of DNA in bovine blood samples. The positive results obtained at low DNA concen-

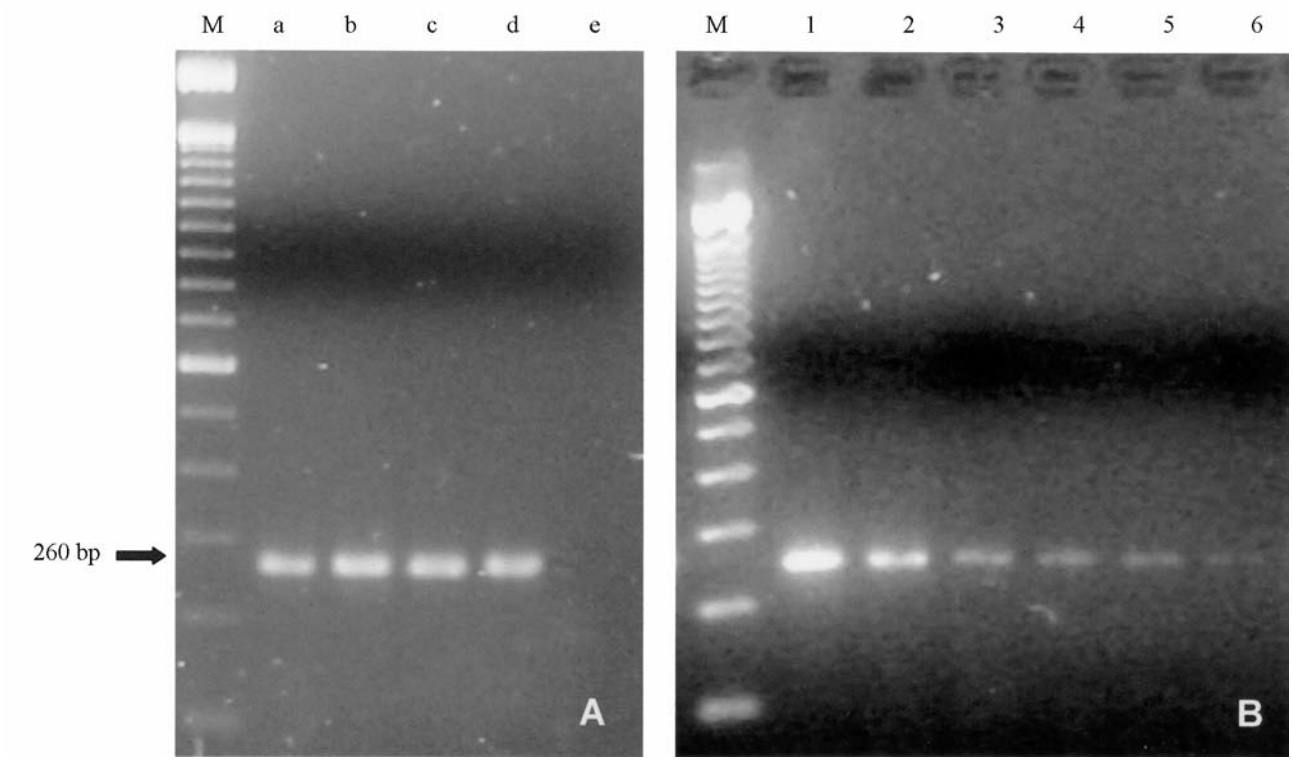

Figure 1 - (A) PCR amplification patterns of bovine DNA using *TSPY*-specific primers. The solid arrowhead indicates the 260 bp Y-specific sequence present in Nelore males (a, b, c, d). This sequence is absent in Nelore females (lane e). M indicates molecular weight marker (100 bp ladder). (B) PCR amplification patterns for various dilutions of DNA from males. The lane numbering (1, 2, 3, 4, 5, and 6) corresponds to the dilutions described in Table 1.

trations probably reflected the elevated copy number for this gene and showed that *TSPY* is potentially useful for sexing cattle, and possibly also for screening bovine embryos. To our knowledge, this is the first study to demonstrate the usefulness of the *TSPY* gene for sexing cattle.

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