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Full Length Research Paper

Complete nucleotide sequencing, SNP identification and characterization of SRY gene in Indian Sangamneri goat

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The SRY gene is responsible for testis determination in mammals. It codes for a protein with a DNA binding domain similar to those found in high-mobility group protein (HMG box). Mutation in this gene leads to Gonadal Dysgenesis an abnormal sexual phenotype described in human, cattle, horse, and river buffalo. Here we report PCR-SSCP analysis and sequencing of coding region of SRY gene in Sangamneri goat (*Capra hircus*) using 40 male goats (bucks) samples. Polymorphism was detected in only one sample indicating that a major portion of SRY gene is highly conserved in this breed.

Key words: Goat, HMG box, SSCP, SRY gene.

INTRODUCTION

India has 20 well-defined breeds of goat (*Capra hircus*) representing a wide spectrum of genetic variability. The genetic variability of indigenous breeds is important for conserving precious and irreplaceable genetic resource that may be applied to new productive demands. Sangamneri goat (*C. hircus*) is found in the Poona and Ahmednagar districts of Maharashtra (Acharya, 1982). It's a medium-sized animals with body color may be white, black or brown, with spots of the other colors. Sex determination in humans depends on the action of a testis-determining factor encoded by the SRY gene consisting of a single exon. The SRY gene, located on the Y chromosome, is responsible for sex determination in mammals (Sinclair et al., 1990) and encodes a protein of 204 amino acids. The SRY gene contains a centrally located region termed the high mobility group (HMG) box, which shows high sequence of conservation between species. This characteristic of the SRY-HMG box region make it an ideal target for development of DNA-based sex determination tests (Prashant et al., 2008). The human Y chromosome is reported to have about 76 protein-coding genes, but only 27 distinct proteins have been identified so far. Mutation in this gene leads to XY Gonadal Dysgenesis (XY GD). The XY GD phenotype has been described in humans (Cohen and Shaw, 1965), horses (Power, 1986), cattle (Kawakura et al., 1996) and river buffalo (Iannuzzi et al., 2001). The XY GD phenotype ascribed to SRY mutations has been reported in humans (Berta et al., 1990), horses (Pailhoux et al., 1995) and cattle (Kawakura et al., 1996). Nevertheless, in human only 15–20% XY GD cases are due to SRY mutations (McElreavey, 1996), when the major part of those can be referred to other known or unknown genetic factors (Veitia et al., 2001). Up until now, only limited studies have been undertaken to explore SRY gene sequence polymorphisms in goat. In SRY 50 flanking region and four intervals of high homology regions have been identified by comparison of human, bovine, pig, goat and mouse genomic sequences (Ross et al., 2008). So far no study has been done to find out genetic variability in the coding region of SRY gene in goat population. The aim of this study was to evaluate the genetic variability in the exonic region of SRY gene using non-radioactive PCR single-stranded conformation polymorphism (PCR-SSCP) leading to sequencing of obtained gel variants in order to develop SNP markers and to establish breeding program based on molecular-marker assisted selection for improvement in Indian goat

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Table 1. Oligonucleotide primers based on *Capra hircus* SRY gene (GenBank D82963).

Primer name	Primer Sequence (5'→ 3')	$MgCl_2$	Ta (^oC)	Product length (bp)
SRY 1F	TGGTAAGAACAGCTTATGAATAGAACG	1.5	53	373
SRY 1R	TCTGTGCCTCCTCAAAGAATG			
SRY 2F	TGGGATACGAGTGGAAAAGG	1.5	55	325
SRY 2R	TTTGTCCAGCTGCTGTGATG			
SRY 3F	TCACAGTCCCTGATTCTAACCAA	1.5	54	316
SRY 3R	AAGAACTGATCAACAGAGGAGCA			

genetic resources.

MATERIALS AND METHODS

We selected 40 genetically unrelated Sangamneri bucks from the native breed; these goats being considered as representative of the existing gene pool of the population. Genomic DNA was isolated from blood as per the methods described by Sambrooks et al. (1989) with minor modifications. Three primers for PCR- SSCP covering the 723 bp long exotic regions was designed on *C. hircus* SRY gene (GenBank D82963) using Primer 3 software (Table 1). BLAST analysis and amplification with female sample was also performed to check the specificity of the designed primer. Optimal annealing temperature of each primer was optimized using a gradient thermocycler (M J Research Inc., MA, USA). The polymerase chain reaction (PCR) was carried out on about 50-100 ng of genomic DNA in 25 µl per reaction volume. The PCR reaction mixture consisted of 200 µM of each dNTPs, 1.5 mM $MgCl_2$, 0.75 unit of Taq DNA polymerase and 40 ng/µl of each primer. PCR amplification was verified by agarose gel electrophoresis. Each PCR product was diluted in denaturing solution (95% formamide, 10 mM NaOH, 0.05% xylene cynol and 0.05% bromophenol blue, 20 mM EDTA) denatured at 85ºC for 13 min, chilled on ice and resolved by SSCP technique to detect the different gel variants in all samples (n = 40) in 1X TBE buffer. The gel was silver-stained (Sambrook and Russell, 2001) and dried on cellophane paper using a Biorad 583 gel dryer. SSCP gel variants were identified from different fragments of the coding region of SRY gene. Purified PCR products for all the gel variants were sequenced using Big Dye Terminator Cycle Sequencing Kit (Applied Biosystem, USA) on an automated Genetic analyzer ABI 3100 (Applied Biosystem, USA). Sequence data were analyzed using Chromas software (Ver 1.45, http://www.technelysium.com.au/chromas.html). Multiple sequence alignment was performed with *ClustalW* program (http://align.genome.jp/).

RESULTS AND DISCUSSION

SSCP analysis for SRY-1 and SRY-2 revealed monomorphic band pattern whereas SRY-3 revealed polymorphic band pattern (Figure 1). In fragment SRY-3 two patterns were detected SRY-3A and SRY-3B with frequency of 97.5 and 2.5%, respectively.

A total 860 bp sequence of SRY gene covering the complete coding region was generated in the present study. Two nucleotide sequences one complete and one partial were deposited under Accession No. EU399905 and EU399906, respectively. The coding region starts from 1 and ends at 723 bp that code for 221 amino acid long SRY protein. In the predicted amino acid sequence of SRY protein the HMG box start at 63rd amino acid and ends at 140th amino acid. BLAST analysis of complete sequence revealed 97% homology of *C. hircus* sequence with *Ovis aries*, 91% with *Bubalus bubalis*, 92% with *Bos grunniens*, *Bos frontalis*, *Bos taurus* and *Bison bison.* Both SSCP patterns of SRY-3 fragment were sequenced and aligned to identify the SNP using *Clustal W* program (Figure 2). In accordance with obtained gel patterns, fragments SRY 1 and SRY 2 were found to be conserved but in fragment SRY-3B, one SNP was present at 179 bp position in non-coding region of the gene where Thymine is replaced by Adenine.

Alignment of coding region of SRY gene of *C. hircus, O. aries, B. taurus, Bos indicus, B. grunniens, B. frontalis, B. bubalis* and *B. bison* showed a high nucleotide and amino acid identity. Our result shows that nucleotide and amino acid sequences are highly conserved along the entire HMG-box region. The Caprine SRY gene is 33 bp longer than the Bovidae SRY gene sequence. SRY gene sequence of sheep and goat show a much higher level of sequence similarity as they belong to same sub family (Payer and Cotinot, 1993). SRY protein of goat and sheep are very similar with 100 percent sequence identity in HMG box region and having a variation of three amino acids in N-terminus region and eleven amino acids in C-terminus region. This clearly shows that after HMG box, N-terminus region is best conserved in this protein. High degree of nucleotide and amino acid identity of SRY gene suggest a close relationship between the members of *Bovidae* families which is supported by fact that fertile hybrids were obtained between Bos × Bison, Goat × Sheep, and Yak × Cattle (Basrur et al., 1967; Cribiu et al., 1988). Different SSCP gel variants with minor and major differences were further cross checked by running the gel for those samples and it showed presence of only two haplotypes. So our result shows that SSCP technique is a reproducible technique to detect genetic variability as reported by several authors (Orita et al., 1989; Neibergs et al., 1993; Sheffield et al., 1993; Dubey et al., 2008). Our data is consistent with the previous literature which states that SRY gene and protein is functionally conserved in whole *Bovidae* family (Cheng et al., 2001).

As the SRY gene is present in the non-recombining region of Y-chromosome so the gene sequence can be

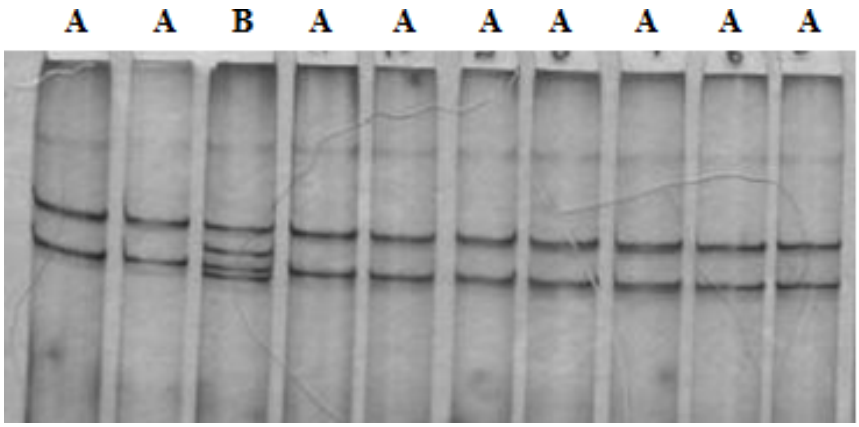

Figure 1. PCR-SSCP band pattern in the SRY-3 fragment on 11% non-denaturing polyacrylamide gel after 4 h of run at 4ºC. The two haplotypes are named alphabetically based on their electrophoretic mobility on the gel. The unique SSCP haplotypes were 316 bp covering exonic and part of 3' UTR region of the SRY gene in Sangamneri goat.

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gi|EU399905.1|      CATCACAGCAGCTGGACAAACCTGGGCCACGATAGGGTAACATTGGATACACGGATTTCC  660
gi|EU399906.1|      ------------------AACCTGGGCCACGATAGGGTAACATTGGATACACGGATTTCC   42
                                      ****************************************

gi|EU399905.1|      GCGGACTTTCCCTTTTACCAAAGCTTAGAGCCTGGGCTTTCTTGCGCTTATGTTCAATAC  720
gi|EU399906.1|      GCGGACTTTCCCTTTTACCAAAGCTTAGAGCCTGGGCTTTCTTGCGCTTATGTTCAATAC  102
                    ************************************************************

gi|EU399905.1|      TGACTTCCTTACTCTCGCTAACAAAGGCACGCTTTATCTCAATTTTACTACAATTTCACC  780
gi|EU399906.1|      TGACTTCCTTACTCTCGCTAACAAAGGCACGCTTTATCTCAATTTTACTACAATTTCACC  162
                    ************************************************************

gi|EU399905.1|      TCCGACTTAATTTTAAACTAAGGCAAATAAGTACATTTAACAAGTAAAGAATTTGGACTT  840
gi|EU399906.1|      TCCGACTTAATTTTAATCTAAGGCAAATAAGTACATTTAACAAGTAAAGAATTTGGACTT  222
                    ***************  ******************************************

gi|EU399905.1|      TCCAAAATAATTGCTCCTCT  860
gi|EU399906.1|      TCCAAAATAATTGCTCCTCT  242
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Figure 2. Comparative alignment of PCR-SSCP haplotypes sequence of SRY-3B (EU399906) and complete SRY gene sequence (SRY-3B: EU399905) using *ClustalW* program (http://align.genome.jp/).

used to analyze and confirm evolutionary divergence time between different goat breeds and other closely related species (Stumpf and Goldstein, 2001; Kikkawa et al., 2003; Parma et al., 2004). In this paper we are reporting complete nucleotide sequence, molecular characterization and one SNP identification in the non-coding region of SRY gene. This baseline SNP data could be utilized for correlation study of semen quality traits of male goats.

These three primers designed in this study were also used to check the cross amplification in both male and female DNA samples of other bovidae species viz. goat, sheep, cattle and buffalo. Primer SRY 2F and SRY 2R were found working in male samples of all the four species which revealed that this primer can be used for sexing in the above species. No SRY-related gene (Sox3 or Sox9) was amplified in female samples proving the specificity of designed primer. The control of the sex ratio is potentially of great commercial value to livestock industry, especially in the dairy and meat sector. Thus it could find important application for DNA based sexing in *Bovidae* family.

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