



Y-STR INRA189 polymorphisms in Chinese yak breeds

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ABSTRACT. To further explore Y-STR INRA189 polymorphisms in the yak, and to determine the genetic differences among yak breeds, genotyping analysis of INRA189 in 102 male yak individuals from three yak breeds in Qinghai Province of China was performed. Genotyping revealed the presence of four alleles, with sizes of 149, 155, 157, and 159 bp, respectively. Of these, the 157-bp allele, which was found with the highest frequency in the three yak breeds, was the dominant allele. Interestingly, the 149-bp allele was only detected in the Gaoyuan breed, and the 159-bp allele was only found in the Huanhu and Datong breeds. Only the 157- and 155-bp alleles were found in all three yak breeds. Taking the three yak breeds as a single population, the frequency of these four alleles was 0.0294, 0.0686, 0.8628, and 0.0392, respectively. The average polymorphism information content in the three yak breeds was 0.2379, indicating that the INRA189 was a low polymorphic Y-STR marker in yak.

Key words: Yak; Polymorphism; Y-STR; INRA189

INTRODUCTION

Yak (*Bos grunniens*) is a bovine species that lives on the Qinghai-Tibetan Plateau and its adjacent territories, which are at high altitudes (2000-5000 m) and are characterized by low atmospheric oxygen and low temperatures. This species can provide pastoralists and agropastoralists living in these areas, mainly the Tibetans, with products (milk, meat, hair, and hides, and manure for fuel) and services (draught, packing, and riding) (Wiener et al., 2003). In China, there are 12 officially recognized yak breeds and around 14 million individuals (Wiener et al., 2003). In Qinghai province, there are three yak breeds, including two indigenous breeds (Gaoyuan and Huanhu) and one improved breed (Datong). Currently, there have been few studies on Y-chromosome markers in the yak (Li et al., 2014). Previous genetic analyses indicated that INRA189 is a yak Y-chromosome-specific microsatellite (Y-STR), which has 2-3 alleles in partial Chinese yak populations and in the Swiss yak population (Han et al., 2000; Nguyen et al., 2005). However, until now, no information on the INRA189 marker is available that can be used to characterize genetic differences between the three yak breeds in Qinghai. The principal aims of this study were to characterize Y-STR INRA189 marker polymorphisms in three yak breeds from Qinghai province and to determine paternal genetic differences.

MATERIAL AND METHODS

Primer sequence

A pair of primers was employed as previously described (Kappes et al., 1997). The forward primer was labeled at the 5' end with HEX fluorescent dye (Sangon, Shanghai, China). The sequences were as follows: Forward primer: 5'-TTT TGT TTC CCG TGC TGA G-3'; Reverse primer: 5'-GAA CCT CGT CTC CTT GTA GCC-3'.

Sampling, DNA extraction, and polymerase chain reaction (PCR) conditions

A total of 102 male individuals, comprising 34 Gaoyuan (GY) yaks collected in Qumalai county, 29 Huanhu (HH) yaks collected in Gonghe county, and 39 Datong (DT) yaks collected at the Datong yak breeding farm of Qinghai province, China, were used for INRA189 genotype analysis. In addition, 10 female Gaoyuan (GY) yaks obtained from the same sampling sites were used as negative controls. Genomic DNA was extracted from blood using a phenol-chloroform protocol followed by an ethanol precipitation step. The PCR reaction mixture contained 50-100 ng yak genomic DNA, 10 μ M each primer, 2X Multiplex PCR Master Mix (Qiagen, Shanghai, China) and ddH₂O to a final volume of 10 μ L. The amplification conditions were as follows: 95°C/5 min, followed by 35 cycles at 94°C/30 s, 60 °C/1 min, 72°C/30 s, and final synthesis at 68°C/10 min.

Genotyping and statistical analysis

Amplified products were scanned together with the GS-500 Liz size standard (Sangon, Shanghai, China) in an ABI 3730xl DNA Analyzer (Applied Biosystems) and fragment lengths were scored using GeneMarker v1.97 (<http://www.softgenetics.com/GeneMarker.html>). Genotypes were assigned for each individual based on allele size data. Allele frequencies were estimated using the GENETIX 4.05 software package (Belkhir et al., 2004). The polymorphic information

content (PIC) was calculated using the PIC_CALC 0.1 software (Nagy et al., 2012) to estimate the informativeness of the microsatellite marker.

RESULTS AND DISCUSSION

In our study, the INRA189 marker was only identified in male yaks, which is consistent with the results of previous studies (Han et al., 2000; Nguyen et al., 2005). These data indicate that INRA189 is a yak Y-STR marker, which can be used in future studies to characterize male-mediated genetic events, paternal genetic diversity, and the origin and evolution of yak breeds.

Previously, Han et al. (2000) and Nguyen et al. (2005) indicated that there are 2-3 INRA189 alleles in partial yak populations. However, in the present study, the results obtained by genotyping INRA189 in 102 male yak individuals revealed the presence of four alleles, with sizes of 149, 155, 157, and 159 bp, respectively (Figure 1). Of these, the allele containing 157 bp was found at the highest frequency in the three yak breeds studied, and is therefore the dominant allele. Interestingly, the allele containing 149 bp was only detected in the Gaoyuan breed, and the 159-bp allele was only found in the Huanhu and Datong breeds. Only the 157- and 155-bp alleles were identified in all three yak breeds. Taking the three yak breeds as a single population, the frequency of these four alleles was 0.0294, 0.0686, 0.8628, and 0.0392 (Table 1), respectively. The PIC in the three yak breeds was 0.2379, which indicates that the INRA189 locus has low PIC (PIC < 0.25) and is therefore a lower polymorphic Y-STR marker in the yak.

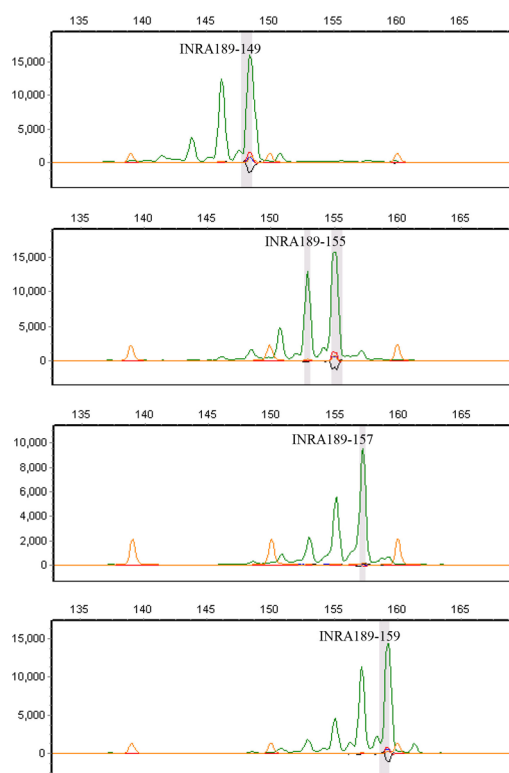


Figure 1. Different alleles of INRA189 in three yak breeds.

Table 1. Allele frequencies of the polymorphic INRA189 marker in three yak breeds.

Breed	Sample No.	INRA189			
		149	155	157	159
Gaoyuan	34	0.0882 (3)	0.0294 (1)	0.8824 (30)	0 (0)
Datong	39	0 (0)	0.0513 (2)	0.8974 (35)	0.0513 (2)
Huanhu	29	0 (0)	0.1379 (4)	0.7931 (23)	0.0690 (2)
Total	102	0.0294 (3)	0.0686 (7)	0.8628 (88)	0.0392 (4)

Numbers in brackets represent the numbers of individuals with each allele.

Conflicts of interest

The authors declare no conflict of interest.

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