# RAPD Variation and Genetic Distances among Tibetan, Inner Mongolia and Liaoning Cashmere Goats

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**ABSTRACT :** Relationship among Tibetan cashmere goats, Inner Mongolia cashmere goats and Liaoning cashmere goats was studied using the technique of random amplified polymorphic DNA (RAPD). One primer and four primer combinations were screened. With the five primers and primer combinations, DNA fragments were amplified from the three breeds. Each breed has 28 samples. According to their RAPD fingerprint maps, the Nei's (1972) standard genetic distance was: 0.0876 between Tibetan cashmere goats and Inner Mongolia cashmere goats, 0.1601 between Tibetan cashmere goats and Liaoning cashmere goats, 0.0803 between the Inner Mongolia cashmere goats and Liaoning cashmere goats. It coincides with their geographic location. The genetic heterogeneity of Tibetan cashmere goats, Inner Mongolia cashmere goats and Liaoning cashmere goats is 0.3266, 0.2622 and 0.2475 respectively. It is also consistent with their development history. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 11 : 1520-1522)*

**Key Words :** Tibetan Cashmere Goats, Inner Mongolia Cashmere Goats, Liaoning Cashmere Goats, RAPD, Genetic Heterogeneity, Genetic Distance

## INTRODUCTION

Cashmere goats originate from Tibet, Middle Asia and other inner Asian areas (Seng, 1997; Jia et al., 1997). They exist extensively in the area of 'Three North' in China. The output of Chinese cashmere down is the highest and the quality is the best of the cashmere downs in the world (Chang, 1996). The Tibet, Inner Mongolia and Liaoning cashmere goats are the most popular in the Chinese cashmere goats. Understanding the germplasm characters and genetic background of the Tibet, Inner Mongolia and Liaoning cashmere goats at the molecular level is becoming increasingly important.

RAPD is a new technique based on PCR (Williams et al., 1990; Welsh and McClelland, 1990). It is a convenient, efficient and sensitive genetic marker of detecting the polymorphism of genome DNA. 10 base (GC-rich) oligonucleotide primers of arbitrary sequence were used in the PCR amplification. And it can get amplification results of several loci. The RAPD technique is extensively used since it appears (Welsh et al., 1991; Kemp and Wolferen, 1992; Rothuizen et al., Mohd-Azmi et al., 2000, 1994; Hwang et al., 2001).

## MATERIALS AND METHODS

### Sample collecting

Blood samples were collected from three goats breeds: The Tibetan were from the Baitu county cashmere goats keeping farm in Ali territory, Tibetan; The Inner Mongolia were from the Inner Mongolia cashmere goats keeping farm; The Liaoning were from the Liaoning cashmere goats keeping farm in Gaizhou city, Liaoning province. All blood samples are unrelated in two generations. Genomic DNA was prepared from peripheral blood leukocyte cells using the procedure described by Li (1997).

### PCR amplification

PCR reactions performed in a 25 μl volume containing 50 ng template DNA, 10×Buffer 2.5 μl, 25 mM $MgCl_2$ 2.0 μl, 2 mM of each dNTP 2 μl, 5 U/μl Taq DNA polymerase 0.3 μl, 10 μM primer 1 μl. each reaction was overlaid with 20 μl mineral oil to prevent evaporation. DNA was amplified in a DNA-Thermocycler programmed for 3 min pre-denaturation at 95°C, followed by 40 cycles. Including 45 seconds at 94°C, 1 min cycle at 37°C, 2 min cycle at 72°C, and then followed by a 5 min extension at 72°C. Blank control was set up for each PCR reaction. All the random primers were recommended by the Operon Company and synthetised by the Shanghai Institute of Cell, the Chinese Academy of Sciences. With the five screened primers and primer combinations, DNA fragments were amplified from the three breeds. Each breed had 28 samples. All the amplification products separated by electrophoresis in 1.5% agarose gels (Tonghai Company, Shanghai) containing 0.05% ethidium bromide, and photographed under UV light. Reproductibility of results was evaluated by replicating the RAPD analysis on DNA samples of all 84 individuals with primers RA59, RA03+RA35, RA04+RA23, RA09+RA35 and RA20+RA33.

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**Data analysis**

Only clear bands of RAPD products on agarose gels were scored. Nei's (1972) standard genetic distance (D) between breeds and genetic heterogeneity within breeds (H) were calculated according to the following equation.

$$D = -\ln\left(\frac{\sum_m\sum_i P_{1mi}P_{2mi}}{\sqrt{\sum_m\sum_i P_{1mi}^2}\sqrt{\sum_m\sum_i P_{2mi}^2}}\right)$$

Where $m$ is number of loci, $i$ is the $i$th locus, $P_{1mi}$ and $P_{2mi}$ are the band frequencies of the $i$th loci in the first and the second population respectively.

$$H = 1 - \sum \frac{2N_{ab}}{(N_a + N_b)}$$

Where $N_a$ and $N_b$ are number of bands for individual a and individual b respectively, $N_{ab}$ is the number of shared bands for individual a and individual b.

## RESULTS

**Primer screening**

There DNA samples of Tibet cashmere goats were used as the amplification templates to carry out the primer screening. Of the 37 arbitrary primers used, the primer of RA59 yield more than four polymorphism bands. Four primer combination (RA03+RA35, RA04+RA23, RA09+RA35, RA20+RA33), whose polymorphism bands are more than four, are screened from 55 primer combinations, which are formed by 11 primers (RA03, RA04, RA09, RA20, RA22, RA23, RA33, RA35, RA41, RA45, RA50) whose amplification bands are more than 8. Those 92 primers and primer combinations yield 560 clear banding pattern of amplified fragments ranging from 300 bp to 5000 bp. Table 1 shows the screening results of the five primers and primer combinations.

**PCR amplification**

61 clear-banding pattern of amplified fragment were produced. 55 (99.16%) of them were polymorphic. Six loci were common. 2,422 (82.7%) were polymorphic in 2926 fragments amplified by the five primer and primer combinations. The frequency of these polymorphic bands ranged from 0.0952 to 0.9048.

Genetic heterogeneities of Tibetan, Inner Mongolia and Liaoning cashmere goats are 0.3266, 0.2622 and 0.2475 respectively. The genetic distances are: 0.0876 between Tibetan cashmere goats and Inner Mongolia cashmere goats, 0.1601 between Tibetan cashmere goats and Liaoning cashmere goats, 0.0803 between the Inner Mongolia cashmere goats and Liaoning cashmere goats.

## DISCUSSION

**Polymorphism of RAPD fingerprint**

Goats are highly resistant to inbreeding depression. The study demonstrates that the frequency of polymorphic bands is too low (0.0833) or too high (0.9167). In order to detect the polymorphism of RAPD markers between goats, a method of combined primers was used. It can meet the requirements of RAPD polymorphic fingerprint. However, it often results in reducing amplification amount. The reason may be the length of the amplified fragments becoming shorter or may be the reduced amount of primers to the amplified fragments. The flaw can be easily ameliorated by increasing the amount of amplified products in electrophoresis.

**Genetic variation of cashmere goats**

The genetic heterogeneity in the populations of Tibetan, Mongolia and liaoning cashmere goats were 0.3266, 0.2622 and 0.2475 respectively. The results reflected that the genetic variations maybe consistent with their selection history. The selection and breeding of Inner Mongolian cashmere goats and Liaoning cashmere goats have been carried out since the 1980s. So genetic variation is lower. On the other hand, the breeding population of Tibetan cashmere goat was formed in 1994, so the selection and

**Table 1.** The screening results of the five primers and primer combinations

| Primer primer combinations | Nucleotides Sequence (5'—3') | Number of RAPD Markers | |
|---|---|---|---|
| | | Total number | Variable number |
| RA59 | CGGGCAACGT | 11 | 5 |
| RA03+RA35 | CGATCGAGGA AAGCTCCCCG | 11 | 4 |
| RA04+RA35 | GCAGAGCATC CTAGCTGACG | 11 | 5 |
| RA09+RA35 | ACTCCGCAGA AAGCTCCCCG | 14 | 6 |
| RA20+RA33 | AATCGATACG TGCGGACGTC | 11 | 4 |

breeding history of it is short and the genetic variation of it is higher (0.3266).

**Genetic distance between cashmere breeds**

In china, Inner Mongolia is located in the middle of Tibetan and Liaoning province. The genetic distances between Tibetan and Inner Mongolia cashmere goats, Tibetan and Liaoning cashmere goats, Inner Mongolia and Liaoning cashmere goats are 0.0816, 0.1601 and 0.0803 respectively. They coincide with their geographic location. The result conforms that goats originate from Tibetan, Middle Asia and other inner Asian areas. Furthermore, we can conclude that the goat's index of Genetic distance and the time of migration from Tibet to the around areas is consistent with its migration distance.

The results described in this paper demonstrate that the technique of RAPD is short of stability, but it is a simple and convenient method. Producing much polymorphic information of target genomes with more primers can compensate the shortcoming of this method. The technique of RAPD is still a good method of studying genetic variation with breed and genetic distance among breeds.

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