Localization of *Sry* gene on Y chromosome of *Muntjac munticus vaginalis* *

ZHANG Yue (张 悦)[1], SHAN Xiangnian (单祥年)[1]**, LIU Ningsheng (刘宁生)[1], LU Xiaoxuan (鲁晓萱)[1], NIE Wenhui (聂文惠)[2], YANG Fengtang (杨凤镫)[2], WANG Jinhuan (王金焕)[2] and CHEN Yuze (陈玉泽)[2]

1 Medical Genetics Research Center, Medical College, Southeast University, Nanjing 210009, China

2 Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China

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Abstract The chromosomes 1, Y_1, Y_2 of *Muntjac munticus vaginalis* were isolated by fluorescence activated chromosome sorting and amplified by degenerate oligonucleotide primed-polymerase chain reaction (DOP-PCR). A primer pair within human *Sry* HMG box was designed and the *Sry* gene of the male *M. m vaginalis* was amplified. The product was cloned and sequenced. The result proved that *Sry* is located on chromosome Y_2, which is the sex-determining chromosome in the male *M. m vaginalis*.

Keywords: ***M. m vaginalis*, *Sry* gene, DOP-PCR, sex.**

The Indian muntjac (*Muntjac munticus vaginalis*) has the lowest diploid chromosome number ($2n = 6$ in the female, 7 in male) so far discovered in mammals[1]. The male *M. m muntjac* has two Y chromosomes. Which one takes part in sex determination or do both of them have the function? This is a puzzling question.

People did not know whether mammalian sex determination depends on the Y chromosome until the late 1950s, and it was proposed that there should be a testis-determining factor on Y chromosome, which determines the genital ridge developing into the testis. In 1990, the SRY gene from sex-determining region of the Y chromosome was isolated in humans by Sinclair et al.[2] And later on it was identified as a conserved gene on the Y chromosome of all animals. In 1991, the mouse homologues *Sry* was shown to express in the genital ridge just before testis differentiation, and its identity as the testis-determining factor was irrefutably demonstrated by the male development of XX mice transgenic for a 14kb DNA containing *Sry*[3].

In this study, we used chromosome sorting, degenerate oligonucleotide primed-polymerase chain reaction (DOP-PCR) and amplification of the *Sry* gene of the male *M. m vaginalis* to localize *Sry* gene on the chromosomes 1, Y_1, $Y \setminus -2$ of *M. m vaginalis*. The results showed that Y_2 is the sex-determining chromosome in the male *M. m vaginalis*.

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** Corresponding author, E-mail: shanxn@jlonline.com

1 Materials and methods

1.1 Cells

The cells of male *M. m vaginalis* derived from embryonic lung were provided by Kunming Institute of Zoology, the Chinese Academy of Sciences.

1.2 Methods

1.2.1 Chromosome preparation and sorting. Harvest and preparation of chromosomes for cytogenetic analyses followed standard procedures. Before harvesting, the cells were treated with colcemid at a final concentration of 0.1 μg/mL, then hypotonized and centrifugated. The pellet was resuspended in 500 μL PAB buffer, then the suspension was stained by the addition of 40 μg/mL chromomycin A3, 2mmol/L $MgSO_4$ and 2 μg/mL of Hoechst 33258 and left on ice for at least 2h. Fifteen minutes prior to flow analysis, Na_2SO_4 and $C_6H_7NaO_7$ were added to the final concentrations of 10 and 25 mmol/L, respectively. The stained chromosome suspensions were sorted on an FACStar Plus flow sorter (Becton Dickinson), and the chromosomes 1, Y_1, Y_2 were isolated[4].

1.2.2 DOP-PCR. The primer used was: 5′-CCGACTCGAGNNNNNNATGTGG-3′(in which N = A, C, G, or T in approximately equal proportions). PCR was carried out in 2.5U *Taq* polymerase, 0.2 mmol/L dNTP, 50 pmol primer, 50 ng template in a volume of 100 μL reaction. After an initial denaturation for 9 min at 94℃, 9 cycles of 94℃ for 1 min, 30℃ for 1.5 min, 72℃ for 3 min proceeded, followed by 30 cycles of 94℃ for 1 min, 62℃ for 1 min, and 72℃ for 3 min. A final extension at 72℃ was increased to 10 min. The size of PCR product was determined by running 3 ~ 5 μL products on a 1% agarose gel.

1.2.3 Amplifying, cloning and sequencing of *Sry* gene of *M. m vaginalis*. The primers designed for *Sry* of *M. m vaginalis* were 5′-TGAAGCGACCCATGAACG-3′ and 5′-CGACGAGGACGATACTTA-3′, which were based on the Panda *Sry* gene sequence[5]. PCR cycling was performed on 4 μL DOP-PCR products of chromosome 1, Y_1, Y_2 respectively, using 2.5 U *Taq* DNA polymerase per 100 μL reaction volume. The terminal cycling included 35 cycles of 1 min denaturation at 94℃, 1 min annealing at 58℃ and 1 min elongation at 72℃. Products of ampification were separated on a 2% agrose gel. A 200bp DNA fragment corresponding to the *Sry* gene was identified as expected, and was excised from the gel. This fragment was then purified and cloned into a PGEM-T plasmid vector[6], and transformed to DH5α. The recombinant clones were identified by color screening. A positive clone was obtained and sequenced.

2 Results and discussion

DOP-PCR amplified uncounted fragments from chromosome 1, Y_1, Y_2 of *M. m vaginali*, which showed a smear on the agarose gel (Fig. 1). The size of products ranged from 200 to 2000 bp.

With the primers designed within human SRY HMG box sequence, the *Sry* gene of the male *M. m vaginalis* was amplified only on chromosome Y_2, but not on chromosome 1 and Y_1(Fig. 2). The sequence of *Sry* HMG box is shown in Figure 3.

DOP - PCR allows essentially random amplification of DNA from any source . It is based on the

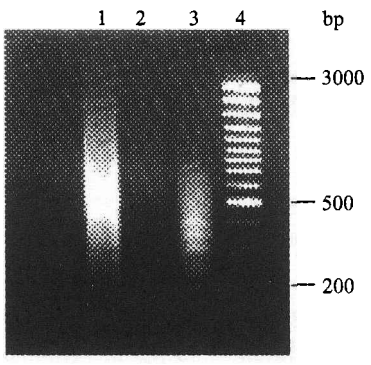

Fig. 1 DOP-PCR products of chromosomes 1, Y_1 and Y_2. Lane 1, Y_1; lane 2, Y_2; lane 3, chromosome 1; lane 4, 100 bp DNA marker.

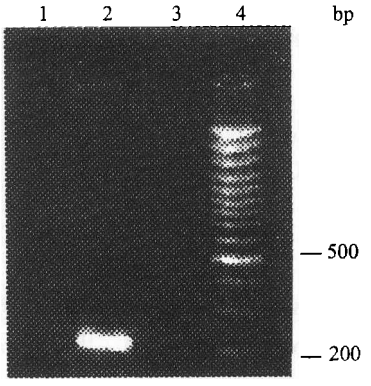

Fig. 2 *Sry* PCR products of chromosomes 1, Y_1 and Y_2. Lane 1, Y_1; lane 2, Y_2; lane 3, chromosome 1; lane 4, 100bp DNA marker.

principle that, at a sufficiently low annealing temperature, only 6 specific bases of 3′ end of the oligonucleotide will prime the reaction, theoretically priming every 4^6 base pairs along the starting DNA. Any specified 6-sequence is therefore likely to occur at a frequency allowing a highly diverse amplification to take place[7]. This is an efficient and convenient technique to amplify a small amount of DNA such as single sorted chromosome.

CCTTCATTGT GTGGTCTCGT GAACGAAGAC GAAAGGTAAC TATAGAGAAT
CCCAAAATGC AAAACTCAGA GATCAGCAAG CAGCTGGGGT ATGAGTGGAA
AAGGCTTACA GATGCTGAAA AGCGCCCATT CTTTGAGGAG GCACAGAGAC
TACTAGCCAT ACACCGAGAC AAATACCCGG GCTAT

Fig. 3 Sequence of Sry HMG box of *Muntjak muntiacus vaginalis*.

Shi et al.[8] have conducted many studies on *Muntjacs* at cytogenetic level. They showed that a high degree diversity of karyotype existed in these animals. The chromosome numbers of female and male *Indian muntjac* are different too. Ma et al.[9] studied synaptonemal complexes in spermatocytes of Chinese *Muntjac Muntiacus reevesi*, Black *Muntjacu M. crinifrons* and Indian *Muntjac M. Muntjak*. They concluded that the sex-determining karyotype of the Indian *Muntjac* was XAXA/XAY.

We proved that *Sry* gene is located on chromosome Y_2, and this chromosome should be the real sex-determining chromosome in the male *M. m vaginalis*.

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