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Sex Identification in Ruminant using SRY and Microsatellite Markers

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ABSTRACT

Multiplex PCR amplification of four microsatellite markers and the SRY was used to identify the sex of the ruminant in the study. Result indicates that the SRY primers are highly specific and can be used as the main basis for sex-identification. The markers of MCM158 and MAF45 are not suitable for sex identification because their low specificity. If the PCR-products of MILVET09 and AE25 are positive but the SRY is negative, then the individual is female. The gene type of MILVET09 and AE25 dose not affect the sex determination of the female. The gene type of MCM158 and MAF45 dose not affect the result of sex-identification, but they can further confirm the male identity. In conclusion, if the PCR-products of SRY and four microsatellite markers are present simultaneously and the gene type of MILVET09 and AE25 is homozygous, then the individual is determined to be normal male, if the PCR-products of four microsatellite markers are present, but the SRY gene is not, then the individual is female. And the gene type of MILVET09 and AE25 does not affect the result of the sex-identification of tested individuals.

Key words: SRY, microsatellite markers, sex-identification, Y chromosome

INTRODUCTION

Animal sex-identification is a great subject in biological field, at the same time it is an important link of embryo project too. According to the needs of production, determining and controlling animal's sex artificially has very important meanings to the domestic animal breeding, actual production and preventing the animal from the chain hereditary disease, also, it has commercial and scientific research value in animal husbandry (Chu, 2001).

The discovery of SRY is a great breakthrough in the research of mammal's sex-determination field in 1990. In recent year, the finding of the SRY location is a biggest progress in the study of the mechanism of sex-determination. With the development of the research in the sex determination mechanism, the method of sex-identification gets synchronous development too, especially the application of the technology of PCR and microsatellite markers which makes the technology of sex-identification achieve practical application stage (Edwards *et al*., 2000; Tzvetkov *et al*., 2010).

In this study the method of amplification the SRY and microsatellite markers on sex chromosome at the same time was adopted (Viger *et al*., 2005), attempted to offer simultaneously the information of the sex-identification and gene type by DNA amplification which improve the accuracy of the sex-identification.

MATERIALS AND METHODS

Animals: Twenty healthy Liangshan semi-fine-wool sheep (Permit No. SCXK 2005·0006), 4 healthy Xinjiang fine-wool sheep (Permit No. SCXK 2008·0001), 5 healthy goats (Permit No. SCXK 2009·0002) and 6 healthy cattle (Permit No. SCXK 2007·0008) were used in this experiment. Committee of science and technology of the Republic of China approves this study. To these experiment animals, provided adequate clean drinking water and food for animal to maintain health and energy and provided enough space, appropriate facilities as well as with similar animal friends, so that the animal could freely express normal habit. Fetched the tissue of ear of the tested individuals, all operations were performed under sodium pentobarbital anehesia and all efforts were made to minimize suffering.

Selection of microsatellite markers, SRY and synthesizing primer: The microsatellite markers MILVET09, AE25 on X chromosome, MCM158, MAF45 and SRY gene on Y chromosome were selected in this experiment. Table 1 showed the nature of selected markers location.

Extraction of DNA: Extracted DNA according to the method of the routine benzene-chloroform.

Genome DNA amplification: Multiple PCR was used in this experiment, 5 pairs of primers were amplified simultaneously. Set up 20 μL reactions of using 1 μL template, 500 nM each primer, 200 μM each dNTP, 2 μL 10 x buffer and 0.2 μL Taq polymerase. Cycling parameters were at 95°C for 8 min, 30 cycles of 95°C for 45 sec, 60°C for 90 sec and 72°C for 60 sec, followed by a single 72°C extension for 10 min. Analyzed PCR-products by Capillary Gel Electrophoresis (De Gortari *et al*., 1997; Weikard *et al*., 2001).

Data analysis: Collection 3.0 software scanned and collected original data, Gene Scan Version 3.1 (PE Biosystem) software automatically analyzed the collected data which could then be imported into Gene type Version 2.5 software for automatic gene typing of alleles.

Table 1: Characterization of SRY gene and four markers

Marker	Chromosome	Size (bp)	Allele	Primer sequences (5'-3')	Resource
SRY	Y	160	1	TGA ACG CTT TCA TTG TGT GGT C	Pig
				GCC AGT AGT CTC TGT GCC TCC T	
MCM158	Y	113	12	TTC CTC TGA GTC TCT GAC AC	Sheep
				CCG AGA TTG AAA TGT AAA TGA G	
MAF45	Y	139	12	ATT GAC ACT TCA GTA AGT TA	Sheep
				CAG ACA CAA CTG AGC AAC TAG C	
MILVET09	X	177	19	AGA CGA TTC CAA ACA CCA GG	Cow
				CTC TCT CGG ATT GCG GTT AG	
AE25	X	100	10	GGC TAA CAC AGC AAT GGG AAT	Sheep
				AAT CTA AGT GTT CAT CAA TA	

RESULTS

Full-automatic analysis the result of the PCR products: Gene software performed gene typing of allele, the sexing of the tested individuals was identified by the SRY gene. The scanning pictures of PCR-products were indicated by electrophoresis area, the primers of selected markers were labeled by fluorescent dyes-6FAM, TET (Fig. 1 and 2) which showed the gene type of the selected markers.

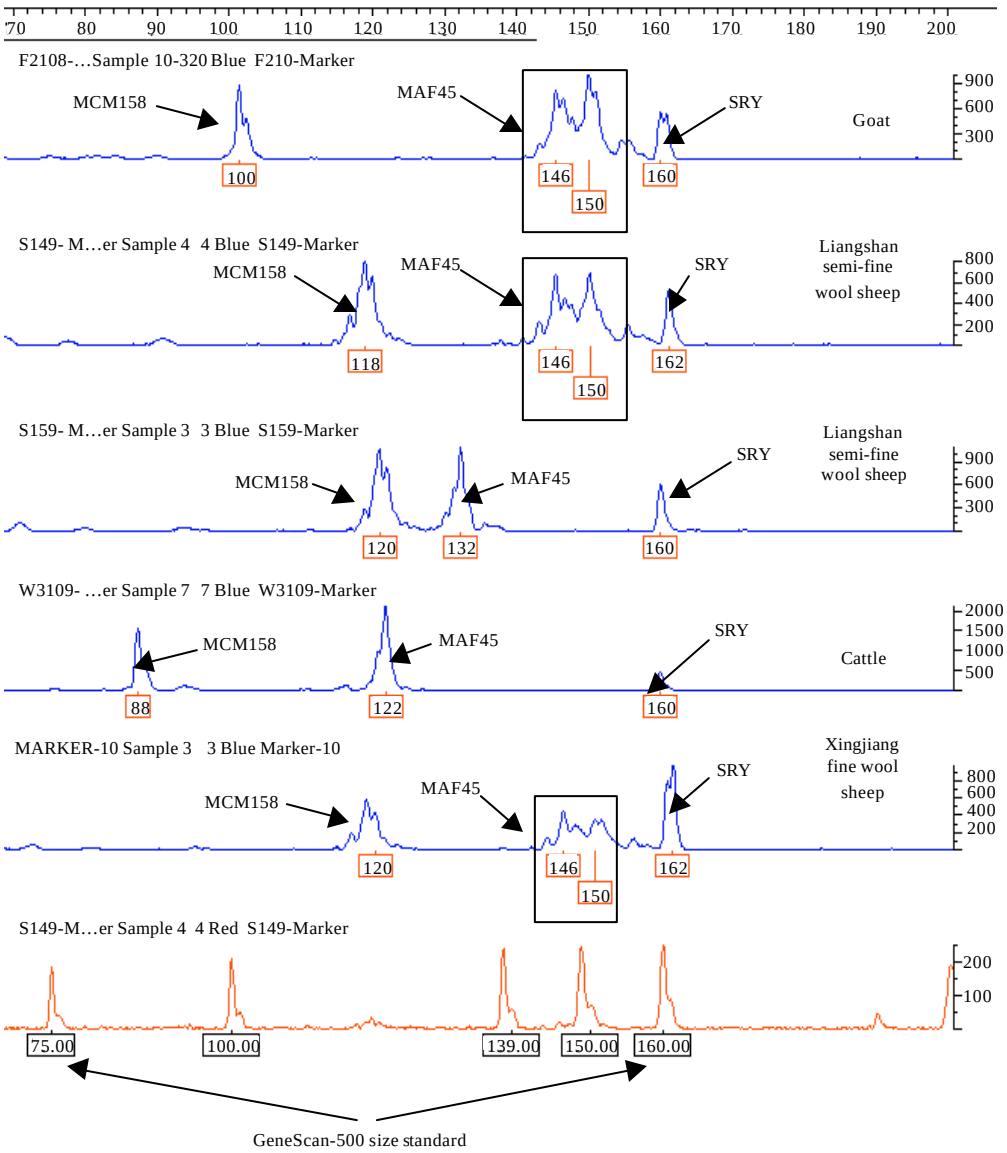

Fig. 1: Comparison results of automatic genotyping of microsatellite markers (MCM158, MAF45) and SRY of goat F2108(M), Liangshan semi-fine wool sheep S149(M), S159(M), Xinjiang fine wool sheep 10(M) and cattle W3109(M)

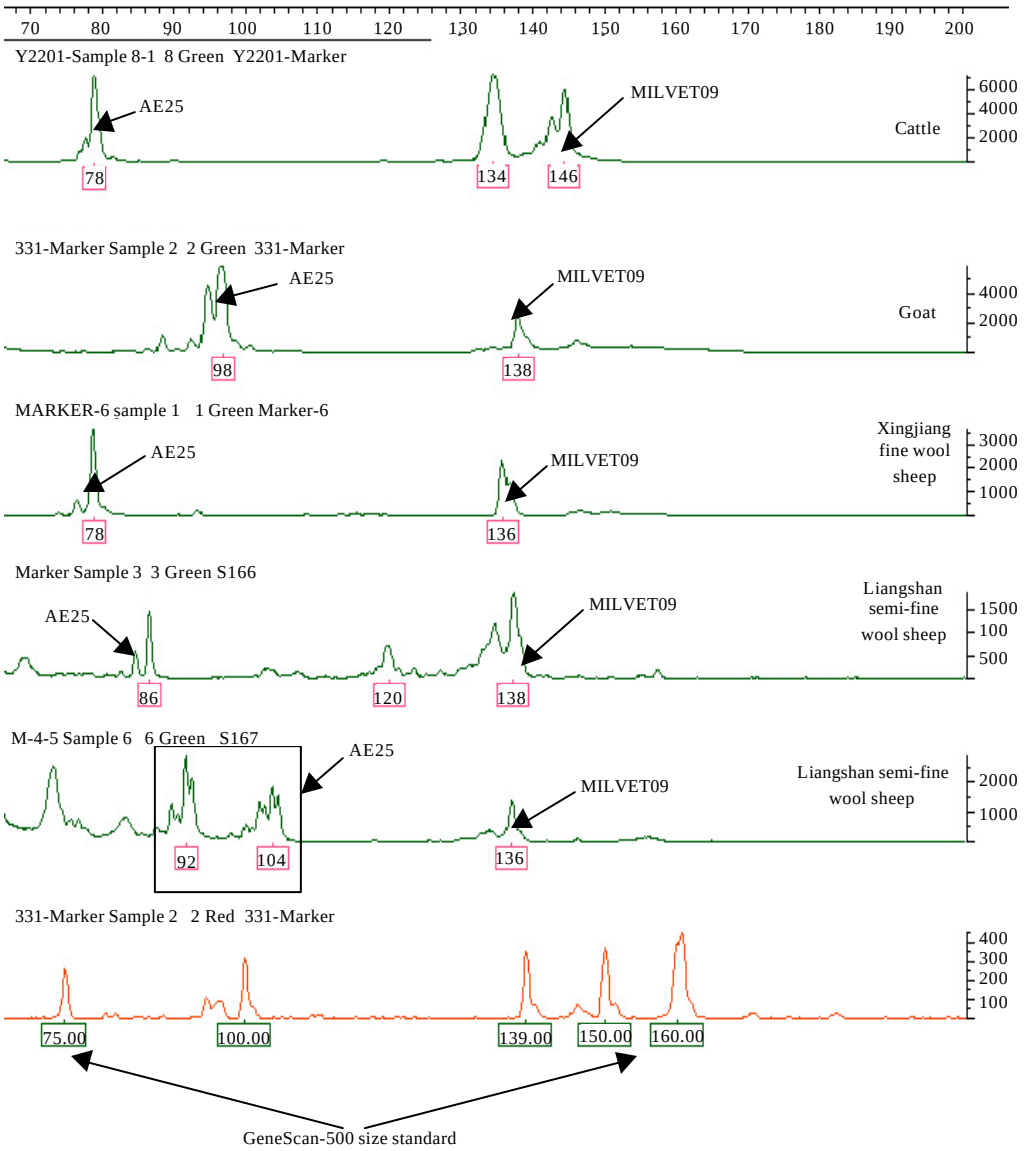

Fig. 2: Comparison results of automatic genotyping of microsatellite markers (MILVET09, AE25) of cattle, Y2201(F), Goat 331(F); Xinjiang fine wool sheep 6(F); Liangshan semi-fine wool sheep S166(F) and S167(F)

Above tested figures showed that the PCR-product of SRY was present for tested males and the MILVET09 and AE25 was homozygous and the MCM158 and MAF45 did not affect the result of the sex-identification of the tested individuals. The PCR-product of SRY was not present for tested females and the selected four microsatellite markers did not affect the result of the sex-identification of the female individuals.

Compare figure of full-automatic analysis: Figure 1 showed the comparison results of the automatic genotyping of selected markers of male individuals. Figure 2 showed the comparison

results of the automatic genotyping of selected markers of female. In figure, the blue peaks are the data of MCM158, MAF45 and SRY gene which primers are labeled by fluorescent dye 6FAM, the green peaks are the data of MILVET09 and AE2 which primers are labeled by fluorescent dye TET.

SRY and four microsatellite markers offered the information of the sex-identification which also indicated the information of the individual difference. The PCR-product of SRY is present in males (Fig. 1), the gene of MCM158 and MAF45 don't affect the sex-identification. Furthermore, the gene type of MILVET09 and AE25 is homozygous in male individuals while their gene type was either homozygous or heterozygous in female individuals.

Genotyping of alleles and sex-identification: In order to obtain the polymorphism information of the sex chromosome, SRY and four microsatellite markers on sex chromosome were amplified by PCR reaction. We amplified the DNA of the tested individuals which based on the success of the amplifying the genome DNA of the known sex samples and we analyzed the initial data by Gene Scan and Gene type software. The results are showed in Table 2.

Table 2: Result of genotyping and sex-identification

		Size of selected markers					
		ChrY			ChrX		
Animal species	Sample	SRY	MCM158	MAF45	MILVET09	AE25	Result of sex-identification
Liangshan	S148	160	112/120	150/154	136/136	94/94	M
semi-fine	S155	-	118/122	136/152	118/138	84/86	F
wool sheep	S149	162	112/112	146/150	138/138	94/94	M
	S150	162	118/122	126/132	138/138	82/82	M
	S154	162	90/90	140/140	138/138	84/84	M
	S156	-	104/120	140/144	120/120	94/94	F
	S158	162	104/108	130/130	120/120	82/82	M
	S159	162	120/120	132/132	136/136	96/96	M
	S160	162	100/100	150/160	138/138	96/96	M
	S163	162	118/118	130/152	138/138	106/106	M
	S164	162	122/122	140/140	120/120	80/80	F
	S166	-	118/122	138/158	138/138	86/86	F
	S167	-	118/122	152/152	136/136	92/104	F
	S168	162	112/122	140/152	138/138	92/92	M
	S181	-	124/124	130/130	120/138	98/98	F
	S186	-	122/126	148/156	138/138	94/94	F
	S187	162	122/122	150/150	138/138	92/92	M
	S191	162	104/122	152/152	138/138	106/106	M
	S194	-	122/122	152/156	120/138	106/106	F
	S209	162	104/110	132/140	120/120	98/98	M
Xingjiang fine	6	-	116/116	146/150	136/136	78/78	F
wool sheep	10	162	118/118	146/150	138/138	96/96	M
	8	-	78/106	128/128	120/120	86/86	F
	5	-	104/122	128/132	122/122	88/98	F

Table 2: Continue

		Size of selected markers					
		ChrY			ChrX		
Animal species	Sample	SRY	MCM158	MAF45	MILVET09	AE25	Result of sex-identification
Goat	BT2109	160	84/90	122/134	120/120	102/102	M
	BY2107	160	96/96	140/156	138/138	96/96	M
	F2108	160	102/102	146/150	136/136	102/102	M
	331	-	94/94	146/146	136/146	96/96	F
	330	-	96/104	128/146	134/136	96/96	F
Cattle	H3109	160	78/90	122/122	138/134	88/88	M
	H3203	-	88/88	122/122	124/138	90/90	F
	H2118	160	122/124	118/118	132/132	90/90	M
	H2201	-	120/120	132/138	146/146	78/78	F
	H1101	160	102/102	128/134	136/136	102/102	M
	H1202	-	114/122	122/136	130/136	78/78	F

Table 2 showed that if the amplification products of the SRY and four microsatellite markers are present simultaneously and the gene type of MILVET09 and AE25 is homozygous, then the tested individuals are normal male. If only the amplification products of four microsatellite markers are present, but the SRY amplification products are not present, then the tested individuals are female. Furthermore, the gene types of MILVET09 and AE25 don't affect the sex-identification of the female. The gene type of MCM158 and MAF45 don't affect the result of the sex-identification.

As shown in Table 2, the fragment length of SRY is 160 or 162 bp which is consilient with Payen and Cotinot (1993) and Pomp *et al.* (1995). It is obvious that SRY is higher conservative among different species of ruminant. The lengths of the four microsatellite markers are different among different species of ruminant or in different individual of the same species ruminant. The PCR-product sizes of the microsatellite markers are different with the published fragment lengths in this study, it is obvious that the PCR-product of the same primer are different among different species of the same animal, so it proves further that the microsatellite markers are with polymorphism.

DISCUSSION

Principle of STR gene: Gene scanning bases on the analysis of fluorescent DNA sequencing, the internal lane size standard was set up, multiple colors testing and genotyping can complete simultaneously (Fan *et al.*, 2003; Marino *et al.*, 1998; Chen *et al.*, 2002). According to this principle, the ABI310 genetic analyzer was utilized to scan the 5 loci on the sex chromosome of the selected tested animals and got some relevant gene data of the 5 loci.

Optimization of the sex-identification method on the molecule level: At present, the test of the Y-DNA STR have been already applied to the research of the anthropology and identifying the male individual extensively, but it is not suitable for the sex-identification. If the PCR amplification failed, we may be judge by mistake and the STR loci exist the fact of the "null allele" (Van Asch *et al.*, 2010; Van Asch and Amorim, 2012; Stacks and Witte, 1996). Owing to the various kinds of defects above, we adopted the method of amplification the SRY and polymorphism

fragments on X and Y chromosome at the same time (Sullivan *et al*., 1993), attempted to offer simultaneously the information of the sex-identification and by DNA amplification which improve the accuracy of the sex-identification.

Discussion of the selected markers to identify the sex: The microsatellite markers were selected according to the sheep gene linkage map issued newly, there are only five microsatellite markers on Y chromosome of the gene linkage map and this fragment is homologous with X chromosome, so the specificity of the selected markers MCM158 and MAF45 is not good, they can't be used for sex-identification alone. The gene type of the MILVET09 and AE25 on X chromosome does not affect the sex-identification of the female but they are able to further confirm the tested male individuals. To the normal male, the gene type of the MILVET09 and AE25 is homozygous, if the gene type of the MILVET09 and AE25 is heterozygous which the sex chromosome is with dysplasia (XXY), then the individuals can't be retained as the breeding stock. The gene type of MCM158 and MAF45 does not affect the result of the sex-identification.

Discussion of the application prospect of the established method: We selected the five gene loci including four polymorphism microsatellite markers and SRY in the study and the established method is used for not only the sex-identification but also identification which has essential difference with the traditional sex-identification, this will have broad application prospect in the fields of sports competition, financial insurance and so on in the future. Furthermore, the method also can be used in monitoring the animal meat product, sex-differentiation and sex hypoplasia,desease sieving, individual identification and paternity test of the forensic medicine. In addition, it also can be used for analysis the trace of the ancient animals and humanity, so the method inaugurates a new avenue for the archaeology.

CONCLUSION

This study assesses the applicability of a previously described four STR and SRY, further improves the accuracy of the sex-identification. The four STR multiplex showed compatible and reproducible results in selected samples and they are able to further confirm the tested male individuals. Moreover, the established method is used for not only the sex-identification but also identification, this will have broad application prospect in forensic caseworks, the fields of sports competition, financial insurance and so on in the future.

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