An AFLP Male-Specific Marker Detected in 15 Iranian Sheep and Goats Populations

Sayed Ziaeddin Mirhoseini [1], Nejat Badbarin [1], Akbar Khaleghzadegan [1]

[1.] Department of Animal Science, University of Guilan, Guilan, Rasht, Iran
szmirhoseini@gmail.com; mirhosin@guilan.ac.ir

Abstract: This study employed the Amplified Fragment Length Polymorphism (AFLP) approach to searching for sex-specific DNA markers in the genome of the Iranian sheep (*Ovis aries*) and goats (*Capra hircus*) populations. Among AFLP primers used to determining sex specific markers, one of them, E42/T32, produce a 100 bp DNA fragment in sheep populations and a 147 bp DNA fragment in goats populations founded only on tested males. This sex specific band in the PCR gel products was represented in males but none was found in females when the population's genomic DNA samples were amplified with these two primers by PCR. This marker frequency among male sheep and goats were 0 to 92.3% and 80 to 100% respectively. The size of the marker was 100 bp and 147 bp in sheep and goats populations respectively.
[Mirhoseini SZ, Badbarin N, Khaleghzadegan A. **An AFLP Male-Specific Marker Detected in 15 Iranian Sheep and Goats Populations**. *Life Sci J* 2012;9(3):2048-2052] (ISSN:1097-8135). http://www.lifesciencesite.com. 296

Keywords: Iranian sheep and goats populations; Male specific marker; AFLP

1. Introduction

Sex determination using genomic DNA from several origins is often an important tool in routine genotyping. Embryo sexing before freezing or transfer into recipient ewes and goats is of considerable economic advantage. Embryo transfer will be more efficient if it could be possible to know the sex of embryos before transfer or freezing. Since reliability is the most important concern in sex determination, PCR is one suitable method that meets this criterion and is rapid, inexpensive and highly sensitive (Dervishi et al., 2008). In addition, sex determination of domestic animal meat has received great attention in recent years. An example exists in beef, where male beef is designated to be of higher quality than cow or heifer meat, and therefore yields higher prices. To avoid unfair competition and to assure consumers of accurate labeling, it is necessary to develop reliable methods for determining the gender of the meats (Bai et al., 2010). Appa Rao et al (1995) developed a method for accurate identification of male and female raw meats in cattle, buffalo, sheep and goat using the PCR technique.

AFLP technique first described by Vos et al (1995), is one of the most powerful DNA fingerprinting technique that could be used for genome studying with any source and complexity. This method was used for studying many investigations including evolution, classification, assessing genetic diversity, construction QTL maps, studying family relationship, assessing population genetic parameters and conservation of genetic pools in animals (Mueller et al., 1999). The AFLP technique provides an efficient marker system for revealing polymorphic loci and for linkage map construction. An AFLP approach in combination with a sex type pool strategy resulted in a quick analysis for identifying sex-linked loci in rainbow trout. Similar approaches may be useful for molecular mapping of markers linked to genes of interest in other species (Felip et al., 2005).

There are 27 native sheep and 3 wild sheep populations in Iran (Valdez et al., 1978). Goats are the most adaptable and widely spread ungulate livestock spices in the world. They have played a central role in the Neolithic agricultural revolution and spread of human civilization (Marsan et al., 2002). In most farms in Iran, sheep and goats keep together in the same herd, so in this study we use AFLP technique to find sex specific markers for both sheep and goat species together.

Sex identification at immature stages may be done using molecular genetic markers [e.g. goat (Shi et al., 2008), sheep (Dervishi et al., 2008), pig (Horng and Huang, 2003), yak (Bai et al., 2010), Giant tiger shrimp (Khamnamtong et al., 2006), Giant Catfish (Sriphairoj et al., 2007), rainbow trout (Felip et al., 2005)]. Among which the AFLP approach has been especially widely used due to the simplicity and reproducibility of the procedure (Griffiths and Orr, 1999). Therefore, AFLP was used in this study aiming at identifying markers specific to each sex of the Iranian sheep and goats populations.

2. Material and Methods

Blood samples were taken from 10 sheep populations including Torki-Ghashghaei, Lori-Bakhtiari, Makuei, Grey-Shiraz, Zandi, Naeini, Moghani, Kalakui, Shal, Taleshi, and 6 goat populations including Naeini, Torki, Abadeh, Khalkhali, Taleshi and Ghazvini (Table 3). Each sample was taken from local populations randomly.

Genomic DNA was isolated from whole blood by the modified salting out method. The concentration of extracted DNA was estimated using a knowing standard DNA (λ DNA) on agarose gels. The procedure adopted for the production and detection of *Eco*RI/*Taq*I AFLP markers was that described in Ajmone-Marsan *et al.* (1997). Table 1 shows *Eco*RI and *Taq*I adapters and used primers were used in the study. Individual goats were assayed with seven highly polymorphic AFLP primer pairs, selected from a 24 combinations previously tested on five individuals (Table 2).

The 25 µl PCR preamplification reaction mixtures were added to 2 µl of diluted 1:5 H_2O and ligated DNA. The preamplification reaction mixture contains 14.8 µl H_2O, 50ng/µl T01 or T02, 50 ng/µl E01, 0.2 mM dNTPs, 10X PCR buffer, 2mM $Mgcl_2$ and 5 u/µl Taq DNA polymerase. After the preamplification reaction, the reaction mixture was diluted 1:5 with double distilled H_2O. The 10 µl PCR selective amplification mixture contained 1 µl of product from the diluted preamplification reaction, 25 ng/µl of both selective primers, 5 u/µl of Taq DNA polymerase, 0.2 mM of dNTPs, 2mM of $Mgcl_2$ and 1x PCR buffer. All amplification reactions were performed as touch down reaction condition. The touch-down PCR conditions were as follows: (30 s at 94°C and 30 s at 65°C followed by 12 cycles with an annealing temperature decreased by 0.7°C per cycle, and 60 s at 72°C), 23 cycles of normal PCR (30 s at 94°C, 30 s at 56°C, and 60 s at 72°C). The products were analyzed on a 6% polyacrylamide sequencing gel in TBE buffer and silver staining method. For DNA visualization, a silver staining protocol with some modification was used.

3. Results

An AFLP approach was used for identification of sex-linked markers in 366 Iranian sheep and 237 goats populations (Table 3). Seven primer combinations assayed revealed 235 and 299 AFLP bands on sheep and goats population respectively. The length of the bands in AFLP fingerprinting was between 50 bp and 600 bp. Several main bands of AFLP fingerprinting of every animal were found. There were some minor bands in many lanes of the individuals tested. This indicated that genetic material from the same population of animals was similar, although not entirely homogenous. In the sheep populations 121 polymorphism bands and in the goat's population 78 polymorphism bands was found, and 114 and 221 bands were homogenous in sheep and goats respectively.

We did not find any bands that were abundant in females but not in males. While one primer pair (E42/T32) gave a 100 and 147 bp fragment in sheep and goats populations respectively that was highly abundant in male samples but not in females, after screening DNA samples (Fig. 1 and 2) we refer to these band as male marker. This band was not found in any female, and its frequency among male sheep and goats were 0 to 92.3% and 80 to 100% respectively (Table 3). These bands also were polymorph among sheep and goats populations with ranged from 100 to 147 base pair (Fig. 1 and 2).

4. Discussions

During the last few decades, based on PCR technique, several different methodologies were developed for determining the gender of domestic animal. Appa Rao et al (1995) developed a simple and reliable method for accurate identification of male and female raw meats in cattle, buffalo, sheep and goat using PCR technique. The PCR assay was conducted on genomic DNA extracted from raw muscle tissue of male and female animals. The method has been found to be accurate, reliable and quick. AFLP analysis is an effective technique for characterizing the chromosomal location of a gene of interest for the ultimate goal of isolation of the gene because a large number of loci can be scored in each primer combination used (Vos et al., 1995).

The result showed that E42/T32 primers could be used for a PCR based sexing technique in these populations. These PCR primers amplified more reliable marker that can be used in sex identification. During the screen for sex-specific markers, no population specific markers were identified. It could be because of any selection within these populations and uncontrolled crossbreeding.

Genetic sex in mammals is normally determined by presence of either XX or XY chromosomes, since genes determining sex are normally in the correct chromosomal locations. Sry (sex determining region on the Y chromosome) is the gene that encodes the testis determining factor in mammals. Thus, in mammalian sex determination, it is expected that XY animals, having the Sry gene, will develop testes and that XX animals, having no Sry gene, will develop ovaries (Baker, 2006). We suppose that this sex specific marker is located on the holandric part of Y chromosome. This band was polymorph in all males of sheep populations but it was homogenous in three goat populations including Torki, Abade and Khalkhali. The location of the sex locus on the Y-chromosome of these populations needs to be further analyzed. A larger number of molecular markers mapping on both sides of this gene may finally help to elucidate this feature on the Y-chromosome.

Table 1. Adapters and primer used in AFLP analysis

	Name	Sequence
Adapter *TaqI*	*Taq* top strand	5'-GACGATGAGTCCTGAC
	Taq bottom strand	5'-CGGTCAGGACTCAT
Adapter *EcoRI*	*Eco* top strand	5'-CTCGTAGACTGCGTACC
	Eco bottom strand	5'-AATTGGTACGCAGTCTAC
Pre amplification primer *EcoRI*	E01	5'-GAC TGC GTA CCA ATT CA
Pre amplification primer *TaqI*	T01	5'-GAT GAG TCC TGA CCG AA
	T02	5'-GAT GAG TCC TGA CCG AC
Selective primer *EcoRI*	E31	5´-GAC TGC GTA CCA ATT C AAA
	E32	5´-GAC TGC GTA CCA ATT C AAC
	E35	5´-GAC TGC GTA CCA ATT C ACA
	E42	5´-GAC TGC GTA CCA ATT C AGT
	E43	5´-GAC TGC GTA CCA ATT C ATA
	E45	5´-GAC TGC GTA CCA ATT C ATG
Selective primer *TaqI*	T32	5´-GAT GAG TCC TGA CCG A AAC
	T33	5´-GAT GAG TCC TGA CCG A AAG
	T48	5´-GAT GAG TCC TGA CCG A CAC
	T50	5´-GAT GAG TCC TGA CCG A CAT

Table 2. Seven primers used for selective amplification

	Pre amplification with E01/T01		Pre amplification with E01/T02	
Primers	T32	T33	T48	T50
E31	E31/T32	E31/T33	**E31/T48**	E31/T50
E32	E32/T32	E32/T33	E32/T48	E32/T50
E35	E35/T32	**E35/T33**	**E35/T48**	E35/T50
E42	**E42/T32**	E42/T33	E42/T48	E42/T50
E43	**E43/T32**	**E43/T33**	E43/T48	E43/T50
E45	**E45/T32**	E45/T33	E45/T48	E45/T50

Table 3. Number of males and females, males, males with specific band and Polymorphism percent of the male specific marker is listed as a proportion of individuals having that marker.

populations		Number of males and females	Number of males	Number of males with specific band	Polymorphism percent (%)
Sheep's breed	Torki-Ghashghaei	15	13	12	92
	Lori-Bakhtiari	40	5	4	80
	Makuei	40	13	7	54
	Grey-Shiraz	15	13	6	46
	Zandi	40	10	3	30
	Naeini	40	20	5	25
	Moghani	40	12	2	17
	Kalakui	51	20	2	10
	Shal	45	13	1	8
	Taleshi	40	10	0	0
Goat's breed	Naeini	42	10	9	90
	Torki	43	12	12	100
	Abadeh	39	16	16	100
	Khalkhali	41	11	11	100
	Taleshi	32	11	10	91
	Ghazvini	40	10	8	80
Total		603	199	108	54

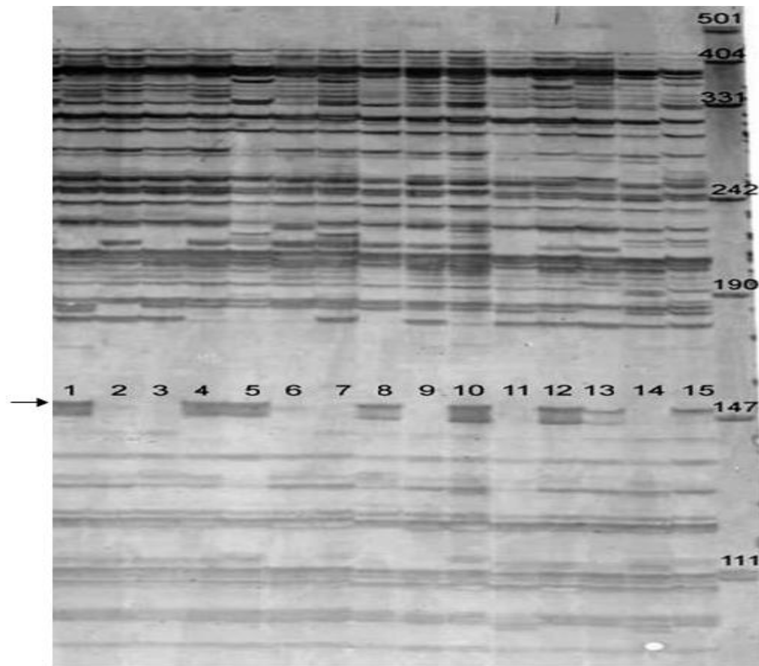

Figure 1. A polyacrylamide gel electrophoresis showing AFLP products of male specific marker in Naeini goat population using E42/T32 primer, with Ladder 1Kb in right. Lanes 1, 4, 5, 8, 10, 12, 13 and 15 are male's samples and Lanes 2, 3, 6, 7, 9, 11 and 14 are female's samples. An arrowhead indicates a candidate sex-specific AFLP marker.

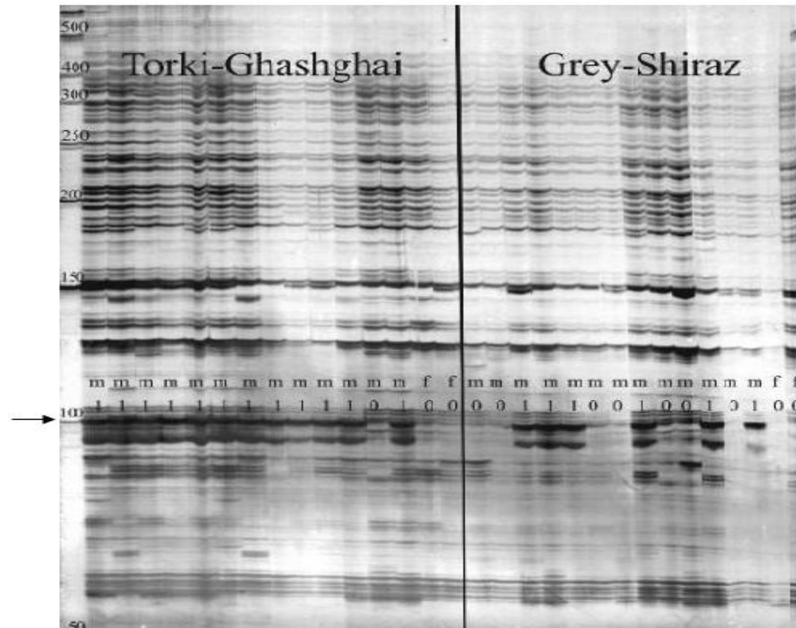

Figure 2. A polyacrylamide gel electrophoresis showing AFLP products of male specific marker in Torki-ghashghaei and Grey-Shiraz sheep populations using E42/T32 primer, with Ladder 1Kb in right. Lanes indicated with m are male's samples and Lanes indicated with f are female's samples. An arrowhead indicates a candidate sex-specific AFLP marker.

It would be interesting to investigate further the structure of the male-specific sequence in these populations. Testing a larger number of individuals is important in order to estimate more correctly the sex specificity of our marker. Screening a larger number of markers may be required to find a more tightly linked one. If sex specific AFLP markers will be verified with more individuals, we plan to convert them to SCAR markers (Sequence Characterized Amplified Regions) to increase the reliability and speed of the testing. In conclusion, novel male specific DNA sequences in Iranian sheep and goats populations could be obtained using a AFLP fingerprinting. The sex of these populations could also be easily and effectively determined using the PCR technique.

Acknowledgements:

This work was supported by Agricultural Biotechnology Research Institute of North of Iran.

Corresponding Author:
Dr. Seyed Ziaeddin Mirhoseini
Department of Animal Science
University of Guilan. Guilan,
Rasht, Iran
E-mail:szmirhoseini@gmail.com;
mirhosin@guilan.-ac.ir

References

1. Dervishi E, Martinez-Royo A, Snchez P, Alabart JL, Cocero MJ, Folch J. Reliability of sex determination in ovine embryos using amelogenin gene (AMEL). Heriogenology. 2008. 70:241-247.
2. Bai WL, Yin RH, Zhao SJ, Li C, Ma ZJ, Yin RL, Luo GB, Zhao ZH. A PCR assay for sex determination of yak (*Bos grunniens*) meat by amplification of the male-specific SRY gene. Food Control. 2010. 21:726-731.
3. Appa Rao KBC, Kesava Rao V, Kowale BN, Totey SM. Sex-specific identification of raw meat from cattle, buffalo, sheep and goat. Meat Science. 1995. 39:123-126.
4. Vos P, Hogers R, Bleeker M, Reijans M, Lee T, Hornes M, Frijters A, Peleman J, Kuiper M, Zabeau M. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research. 1995. 23:4407-4414.
5. Mueller UG, Wolfenbarger LL. AFLP genotyping and fingerprinting. Tree. 1999. 14:389-394.
6. Felip A, Young WP, Wheeler PA, Thorgaard GH. An AFLP-based approach for the identification of sex-linked markers in rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 2005. 247:35-43.
7. Valdez R, Nadler CF, Bunch TD. Evolution of wild sheep in Iran. Evolution. 1978. 32:56-72.
8. Marsan PA, Negrini R, Milanesi E, Crepaldi P, Cicogna M, Zagdsuren Y, Ertugrul O, Luikart G, Taberlet P. Geographic structure in goat diversity as revealed by AFLP molecular markers. 7th World Congress on Genetics Applied to Livestock Production, 2002. Montpellier, France.
9. Shi L, Yue W, Ren Y, Lei F, Zhao J. Sex determination in goat by amplification of the HMG box using duplex PCR. Animal Reproduction Science. 2008. 105:398-403.
10. Horng YM, Huang MC. Male-specific DNA sequences in pigs. Theriogenology. 2003. 59:841-848.
11. Khamnamtong B, Thumrungtanakit S, Klinbunga S, Aoki T, Hirono I, Menasveta P. Identification of Sex-specific Expression Markers in the Giant Tiger Shrimp (*Penaeus monodon*). Journal of Biochemistry and Molecular Biology. 2006. 39:37-45.
12. Sriphairoj K, Kamonrat W, Na-Nakorn U. Genetic aspect in broodstock management of the critically endangered Mekong giant catfish, Pangasianodon gigas in Thailand. Aquaculture. 2007. 264:36-46.
13. Griffiths R, Orr K. The use of amplified fragment length polymorphism (AFLP) in the isolation of sex-specific markers. Molecular Ecology. 1999. 8:671-674.
14. Ajmone Marsan P, Valentini A, Cassandro M, Vecchiotti Antaldi G, Bertoni G, Kuiper M. AFLP™ markers for DNA fingerprinting in cattle. Animal Genetics. 1997. 28: 418-426.
15. Baker JA. Genetics, genomics, and molecular biology of sex determination in small animals. Theriogenology. 2006. 66:1655-1658.

1/26/2012