

Microsatellite panels for parentage testing of Kilis goats reared in Turkey

Mahmut KESKİN^{1*}, Onur YILMAZ², Zuhale GÜNDÜZ¹, Neziha ATA²,

Sabri GÜL¹, İbrahim CEMAL², Orhan KARACA², Süleyman Ercüment ÖNEL³

¹Department of Animal Science, Faculty of Agriculture, Hatay Mustafa Kemal University, Hatay, Turkey

²Department of Animal Science, Faculty of Agriculture, Adnan Menderes University, Aydın, Turkey

³Samandağ Vocational School, Hatay Mustafa Kemal University, Hatay, Turkey

Received: 07.09.2018

Accepted/Published Online: 30.12.2018

Final Version: 12.02.2019

Abstract: The aim of this study was to develop PCR-based suitable microsatellite marker panels for paternity testing and to define pedigree errors in Kilis goats. A total of 137 head of goats were used, consisting of 118 head of kids and 19 head of possible candidate sires. A total of 392 alleles were observed in 22 microsatellite markers. Allele numbers ranged between 12 (SRCRSP7) and 24 (BM1818, INRA0023, and SRCRSP15) with an average of 17.82. The mean value of the effective allele numbers was obtained as 9.44. The overall polymorphic information content value was quite high (0.88). The overall observed (H_o) and expected heterozygosity (H_e) values for all studied loci were 0.89 and 0.89, respectively. Paternity test panels for the Kilis goat population studied were created based on individual probability of exclusion of microsatellites in multiplex groups. Combined probability of exclusion (CPE) values for different panels ranged between 0.745 (Panel-1) and 0.9999 (Panel-22), while the combined identification probability (CPI) values were obtained in the range of 9.81×10^{-3} (Panel-1) to 6.96×10^{-21} (Panel-22). As a result of this study, it can be stated that panels with 0.999 CPE values can be used at the most reasonable cost and with high reliability in paternity tests to be performed in Kilis goats, which can be a reference for other populations.

Key words: Kilis goats, microsatellite, parentage testing, probability of exclusion

1. Introduction

Identification and validation of the biological father of a living thing by methods such as blood group, hemoglobin typing, transferrin typing, or DNA analysis is defined as a paternity test. Pedigree records in animal production farms can be misrecognized for many reasons. Here, paternity tests are used to identify and verify this type of information (1). Because they contain a high level of information, microsatellite markers are used widely in paternity tests to control pedigree records. The high information content of microsatellite markers depends on the number of alleles given by the marker and the frequencies in the corresponding populations (2).

The accuracy of pedigree information in many animal species is one of the most important steps for breeding programs. Pedigree errors have great influence on the answer to the selection in the breeding programs (3). Incorrect selection of male stock candidates in animal breeding programs will result in lower genetic progress than expected (1,4).

In countries such as Turkey, keeping accurate yield and reproductive records in breeding programs for goat

populations raised under extensive condition will increase the degree of precision in the breeding value estimation. In addition, the correct use of paternity tests will contribute to the creation of this information for an unknown population of pedigree information. It will also allow for checking the accuracy of existing pedigree information and correcting the incorrectly recorded information (5). Although paternity test studies are carried out on Turkish native sheep breeds (5,6), there are few studies on paternity testing for Turkish native goat breeds, except in a study carried out in Hair goat (7,8).

The National Sheep and Goat Breeding Project is being implemented in Turkey by the Republic of Turkey Ministry of Food, Agriculture, and Livestock with the cooperation of different universities, research institutes, and sheep and goat breeder associations under the condition of breeders. The Kilis Goat Breeding Project is a subproject of this national project and it has been executed in the Kilis and Gaziantep provinces of Turkey for 7 years. During this project, mating records have been recording by breeders in elite flocks.

The aims of this study are to confirm the pedigree information obtained from mating records in the Kilis

* Correspondence: mkeskin@mku.edu.tr

Goat Breeding Project by paternity tests based on DNA analyses and to develop a panel that can be used with the most reasonable cost and high reliability from 22 microsatellites used in this study since the use of many microsatellite loci in paternity tests is expensive and time-consuming.

2. Materials and methods

2.1. Animal material and DNA isolation

The animal material of this study consisted of 19 head of bucks that were probable candidate sires with 118 head of kids born from hand-mating in the Kilis Goat Breeding Project from 5 different farms with the approval of the Mustafa Kemal University Ethics Committee (MKÜHADYK-2015/10-1). Two months before the date of the mating, the does and bucks were separated from each other and managed separately in farmer conditions. At the time of mating, teaser bucks were used for estrus detection. After this detection, it was recorded which doe was mated by which buck. The numbers of bucks and kids in these 5 farms are given in Table 1.

Using vacutainer tubes containing the anticoagulant K₃-EDTA, blood samples were collected from bucks during the breeding season and from their kids at birth. Genomic DNA was extracted from the blood samples (9,10). Afterward, quality and quantity of DNA was measured using NanoDrop (ND2000, Thermo Scientific, USA).

2.2. Polymerase chain reaction and fragment analysis

In this study, 22 microsatellites loci labeled with fluorescent dye (D2, D3, and D4), as recommended by the FAO (11), were used. PCR amplification was performed for two multiplex groups. The first (M1) and second (M2) multiplex groups consisted of 10 (OarFCB20, INRA0005, INRA0023, ILSTS011, SRCRSP9, SRCRSP15, TCRVB6, OarAE54, INRA0132, and BM1818) and 12 (SRCRSP7, McM0527, CSR0247, INRABERN185, SRCRSP0023, ILSTS0087, SRCRSP0005, DRBP1, INRABERN172, HSC (OLADRB), SRCRSP3, and BM1329) microsatellite markers, respectively. PCR mixtures containing 1X PCR

buffer, 2.0 mM MgCl₂, 0.2 mM dNTPs, 0.1 μM of primers (forward and reverse), 1 U of Taq DNA polymerase (Applied Biological Materials Inc., USA), ~50 ng genomic DNA, and sterile ddH₂O with total volume of 20 μL were prepared for amplification of primer-specific sites in the PCR steps. The touchdown PCR technique, as reported by Hecker and Roux (12), was applied in order to achieve more efficient and rapid DNA amplification in the study (Table 2).

Capillary electrophoresis was performed with a Beckman Coulter GeXP fragment analyzer (Beckman Coulter, Inc., USA) for this separation of fragments.

2.3. Formation of paternity test panels

The microsatellites in each multiplex group were ranked according to their individual probability of exclusion (PE) values and 22 paternity test panels were created according to this procedure in this study (Table 3).

2.4. Statistical analysis

The numbers of alleles (Na), effective alleles (Ne), observed (Ho) and expected (He) heterozygosities, and their mean values for the microsatellite loci used in this study were calculated using the GenAlEx genetic analysis program (13). The Cervus 3.0.3 (14) program was used to calculate polymorphic information content (PIC), probability of exclusion (PE), probability of identity (PI), probability of combined exclusion (CPE), combined probability of identity (CPI), mean proportion of the genotyping individuals (GR), and null allele frequency (F(Null)). The discrimination power of the microsatellites (PD) was determined by the PowerStats v.1.2. program (15).

3. Results

Microsatellite-based genetic polymorphism and paternity analysis statistics for Kilis goats constituting the animal material of this study are given in Table 4. A total of 392 alleles were observed in the 22 microsatellite loci examined. Alleles numbers ranged from 12 (SRCRSP7) to 24 (BM1818, INRA0023, and SRCRSP15) with an average number of 17.82. The mean value of the number of effective alleles was obtained as 9.44.

When the Na, Ne, MNa (the mean number of alleles), and PIC values obtained in this study are examined, it is noteworthy that the microsatellites used have very high polymorphism. The average PIC value, which is an important criterion in the selection of microsatellites, was quite high (0.88). The high PIC value suggests that the microsatellites used in the study can be used effectively in paternity tests. The mean of the expected (He) and observed heterozygosity (Ho) values for all studied locus were 0.89 and 0.89, respectively.

The lowest and the highest values of PE, which is defined as the probability to exclude a random parent pair that is unrelated to the individual, were observed in ILSTS011

Table 1. Distribution of animal material by farms.

Farms	Number of kids	Number of bucks
Farm-1	21	4
Farm-2	30	4
Farm-3	21	4
Farm-4	27	4
Farm-5	19	3
Total	118	19

Table 2. Thermal cycling conditions according to touchdown PCR method.

Multiplex group	First denaturation	Denaturation	Annealing	Extension	Cycle	Final extension
M1 and M2	95 °C (5 min)	95 °C (40 s)	50–60 °C (40 s)	72 °C (60 s)	30	72 °C (10 min)

(0.384) and DRBP1 (0.750) loci, respectively. The higher the PE value, the more non-fathers are excluded.

The PI value, also known as matching probability, which is the probability of two independent samples having the same identical genotype, ranged from 9.34E-03 to 8.75E-02 in this study. The PD, known as the probability that two randomly selected individuals have identical phenotypes or genotypes by chance alone, ranged from 0.890 to 0.981. The higher the discrimination power of a locus, the more efficient it is in discriminating between members of the population. The null allele, which was first introduced by Paetkau and Strobeck (16), causes misreading of the microsatellite peaks. The lowest and highest null allele frequencies were -0.0428 (OarAE54) and 0.0617 (SRCRSP7), respectively.

Information on the correct and incorrect pedigree records according to the results of the paternity tests performed by the microsatellite markers in this study is given in Table 5. As can be seen from Table 5, the average pedigree error rate for five farms was calculated as 3.40%. It can be said that pedigree errors detected are negligible in the population studied.

Paternity test panel statistics, which included the mean number of alleles (MNa), mean polymorphic information content (MPIC), mean expected heterozygosity (MHe), combined probability of exclusion (CPE), combined probability of identity (CPI), and mean proportion of the genotyping individuals (GR), are given in Table 6.

The MNa values ranged between Panel-22 (16.75) and Panel-1 (24.00). The highest and lowest MHe values were observed to be 0.93 (Panel-1, 2, 3, and 11) and 0.88 (Panel-10), respectively. Obtained PIC values from paternity test panels formed varied from 0.87 (Panel-10) to 0.93 (Panel-11). The highest CPE values were obtained from Panel-10 and Panel-22, which were formed by considering the individual PE values of the microsatellites in the multiplex groups. In addition, the CPI values obtained (<0.01) across all the paternity test panels indicated that these panels have high power of individual identification. Mean proportion of the genotyped individuals' value was 97.28% in across all panels studied.

4. Discussion

Obtained MNa and PIC values were significantly higher than those of some other Turkish goat breeds and other

goat breeds raised in different countries (17,18), while these values were lower than those of goat breeds raised in North Africa (19). The findings related to allele numbers indicated a high level of allelic richness in the breed studied. On the other hand, values obtained for PIC showed that the microsatellites used in this study are highly polymorphic. The overall He and Ho were higher than in past studies conducted in different goat breeds (20,21).

Although the obtained average PE value (0.635), which is known as a measure of efficiency in paternity testing, was in the probability of exclusion with some literature (20,22), it was higher than values obtained from other studies (19). This may have been due to the differences in microsatellite loci used in these studies. The high PD value (>0.85) obtained from the loci used in the study is a natural consequence of the high number of alleles obtained from the microsatellite loci. Null alleles, defined as nonamplifiable alleles due to mutations in the PCR binding site, cause only a single allele to peak like a homozygote, thus cause erroneous readings. It was reported by Dakin and Avise (23) that null allele frequencies below 0.20 have no significant effect on paternity tests. When the null allele frequencies obtained are examined, it is seen that the null allele frequency values of 22 microsatellites to be studied are below 0.20. Taking this value into consideration, it has been demonstrated that the studied loci can be safely used in paternity tests.

The obtained low level of pedigree errors does not pose any risk for genetic parameters to be calculated in populations. In addition, the obtained findings from the present study showed that the errors in the pedigree records can be corrected with high accuracy by the paternity tests to be performed by the microsatellite markers. A total of four kid's sires were recorded incorrectly in the pedigree record. These errors may have occurred due to many factors such as paddock overturning, writing the wrong number during mating, and mistyping of lambs at birth.

The mean number of alleles (MNa), mean expected heterozygosity (MHe), and mean polymorphic information content (MPIC) values obtained from the generated paternity test panels were higher than the values in Brazilian goat breeds reported by da Da Silva et al. (18).

The minimum CPE recommended for accurate identification of the true father is 0.999 (24,25). As can be seen from Table 6, the CPE values obtained for the 22

Table 3. Formation of paternity test panels.

Multiplex-1												
Panel	SRCRSP15 (PE = 0.745)	INRA0132 (PE = 0.7229)	TCRVB6 (PE = 0.7199)	BMI1818 (PE = 0.687)	OarFCB20 (PE = 0.647)	SRCRSP9 (PE = 0.622)	INRA0023 (PE = 0.596)	INRA0005 (PE = 0.589)	OarAE54 (PE = 0.574)	ILSTS011 (PE = 0.384)		
Panel-1	✓											
Panel-2	✓	✓										
Panel-3	✓	✓	✓									
Panel-4	✓	✓	✓	✓								
Panel-5	✓	✓	✓	✓	✓							
Panel-6	✓	✓	✓	✓	✓	✓						
Panel-7	✓	✓	✓	✓	✓	✓	✓					
Panel-8	✓	✓	✓	✓	✓	✓	✓	✓				
Panel-9	✓	✓	✓	✓	✓	✓	✓	✓	✓			
Panel-10	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Multiplex-2												
Panel	DRBP1 (PE = 0.750)	INRABERN185 (PE = 0.693)	HSC (PE = 0.690)	INRABERN172 (PE = 0.667)	BMI1329 (PE = 0.659)	SRCRSP0023 (PE = 0.652)	SRCRSP0005 (PE = 0.644)	MCM0527 (PE = 0.642)	CSRD0247 (PE = 0.600)	SRCRSP7 (PE = 0.572)	ILSTS0087 (PE = 0.566)	SRCRSP3 (PE = 0.556)
Panel-11	✓											
Panel-12	✓	✓										
Panel-13	✓	✓	✓									
Panel-14	✓	✓	✓	✓								
Panel-15	✓	✓	✓	✓	✓							
Panel-16	✓	✓	✓	✓	✓	✓						
Panel-17	✓	✓	✓	✓	✓	✓	✓					
Panel-18	✓	✓	✓	✓	✓	✓	✓	✓				
Panel-19	✓	✓	✓	✓	✓	✓	✓	✓	✓			
Panel-20	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Panel-21	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Panel-22	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Table 4. Polymorphism statistics of microsatellite loci.

Loci	N	Na	Ne	Ho	He	PIC	PE	PI	PD	F(Null)
OarAE54	134	18	7.08	0.93	0.86	0.85	0.574	3.26E-02	0.952	-0.0428
INRA0132	133	22	12.35	0.94	0.92	0.91	0.722	1.19E-02	0.973	-0.0126
BM1818	133	24	10.78	0.94	0.91	0.90	0.687	1.57E-02	0.970	-0.0195
OarFCB20	132	17	9.35	0.93	0.89	0.88	0.647	2.05E-02	0.966	-0.0211
INRA0005	132	14	7.77	0.87	0.87	0.86	0.589	2.95E-02	0.961	0.0012
INRA0023	137	24	7.46	0.82	0.87	0.86	0.596	2.76E-02	0.961	0.0303
ILSTS011	132	15	4.33	0.77	0.77	0.74	0.384	8.75E-02	0.890	-0.0049
SRCRSP9	136	16	8.63	0.84	0.88	0.87	0.622	2.41E-02	0.963	0.0243
SRCRSP15	133	24	13.84	0.95	0.93	0.92	0.745	9.82E-03	0.981	-0.0103
TCRVB6	137	17	12.45	0.95	0.92	0.91	0.719	1.22E-02	0.980	-0.0165
SRCRSP3	136	16	6.83	0.88	0.85	0.84	0.556	3.55E-02	0.945	-0.0225
BM1329	136	19	9.77	0.94	0.90	0.89	0.659	1.91E-02	0.968	-0.024
SRCRSP7	134	12	7.43	0.77	0.87	0.85	0.572	3.27E-02	0.959	0.0617
McM0527	128	18	9.19	0.81	0.89	0.88	0.642	2.13E-02	0.971	0.0481
CSRD0247	136	17	8.06	0.81	0.88	0.86	0.600	2.76E-02	0.965	0.0400
INRABERN185	130	18	10.98	0.96	0.91	0.90	0.693	1.48E-02	0.973	-0.0283
SRCRSP0023	135	21	9.42	0.84	0.89	0.89	0.652	2.00E-02	0.974	0.0296
ILSTS0087	135	13	7.18	0.86	0.86	0.85	0.566	3.40E-02	0.951	-0.0009
SRCRSP0005	135	16	9.26	0.91	0.89	0.88	0.644	2.09E-02	0.972	-0.0105
DRBP1	131	19	14.25	0.99	0.93	0.93	0.750	9.34E-03	0.981	-0.0330
INRABERN172	134	14	10.20	0.96	0.90	0.89	0.667	1.79E-02	0.970	-0.0288
HSC(OLADRB)	135	18	11.03	0.96	0.91	0.90	0.690	1.52E-02	0.976	-0.0297
Mean		17.82	9.44	0.89	0.89	0.88				

Na: Number of alleles, Ne: number of effective alleles, Ho: observed heterozygosity, He: expected heterozygosity, PIC: polymorphic information content, PE: probability of exclusion, PI: probability of identification, PD: power of discrimination, F(null): null allele frequency.

Table 5. Percentage of sires whose pedigree records are correct and incorrect.

Farms	Kid number	Mating records		Error rate (%)
		True	False	
Farm-1	21	21	-	0.00
Farm-2	30	29	1	3.00
Farm-3	21	20	1	4.80
Farm-4	27	25	2	7.40
Farm-5	19	19	-	0.00
General	118	114	4	3.40

microsatellites used in this study are within the specified limits. When the CPE values obtained in this study are examined, it is seen that panels formed by six or more microsatellites reached the minimum CPE values reported in the literature. It is an important finding that the CPE values obtained from the paternity test panels formed by six or more microsatellites in the present study are in agreement with the values obtained from paternity test panels formed by eleven microsatellites reported by Luikart et al. (24) and ISAG (26). At the same time, these values were similar to CPE values obtained from paternity test panels consisted of sixteen (18), twelve (27), seventeen (28), and eight (17) microsatellite markers. On the other hand, these values were higher than the values obtained

Table 6. Paternity test parameters for panels formed with increasing numbers of microsatellites.

	Panels	NMP	MNa	MHe	MPIC	CPE	CPI	GR
MG1	Panel-1	1	24.00	0.93	0.92	0.745322	9.8E-03	97.08%
	Panel-2	2	23.00	0.93	0.92	0.929123	1.2E-04	97.08%
	Panel-3	3	21.00	0.93	0.92	0.980057	1.4E-06	98.05%
	Panel-4	4	21.75	0.92	0.91	0.993759	2.24E-08	97.85%
	Panel-5	5	20.80	0.92	0.91	0.997796	4.6E-10	97.52%
	Panel-6	6	20.00	0.91	0.90	0.999168	1.1E-11	97.81%
	Panel-7	7	20.57	0.91	0.90	0.999664	3.1E-13	98.12%
	Panel-8	8	19.75	0.90	0.89	0.999862	9.0E-15	97.90%
	Panel-9	9	19.56	0.90	0.89	0.999941	2.9E-16	97.89%
	Panel-10	10	19.10	0.88	0.87	0.999964	2.6E-17	97.74%
MG2	Panel-11	1	19.00	0.93	0.93	0.750233	9.3E-03	95.62%
	Panel-12	2	18.50	0.92	0.91	0.923253	1.4E-04	95.26%
	Panel-13	3	18.33	0.91	0.91	0.976238	2.1E-06	96.35%
	Panel-14	4	17.25	0.92	0.91	0.992097	3.7E-08	96.72%
	Panel-15	5	17.60	0.91	0.90	0.997307	7.1E-10	97.23%
	Panel-16	6	18.17	0.91	0.90	0.999063	1.4E-11	97.45%
	Panel-17	7	17.86	0.91	0.90	0.999666	3.0E-13	97.60%
	Panel-18	8	17.88	0.91	0.90	0.999881	6.4E-15	97.08%
	Panel-19	9	17.78	0.90	0.89	0.999952	1.8E-16	97.32%
	Panel-20	10	17.20	0.90	0.89	0.999980	5.8E-18	97.37%
	Panel-21	11	16.82	0.90	0.88	0.999991	2.0E-19	97.48%
	Panel-22	12	16.75	0.89	0.88	0.999996	7.0E-21	97.63%
MG1-2	Panel-23	22	17.82	0.89	0.88	0.999999	1.78E-37	97.68%

NMP: Number of microsatellites in the panel, MG: multiplex group, MNa: mean number of alleles, MHe: mean expected heterozygosity, MPIC: mean polymorphic information content, CPE: combined probability of exclusion, CPI: combined probability of identity, GR: mean proportion of the genotyping individuals.

from paternity test panels reported by the MAPA (29). It is believed that panels with a CPE above 0.999 in the presented study may be suitable for use in other goat breeds.

It is clear that increasing the number of microsatellite loci to be used in the panels increases the value of the CPE. However, working with a smaller number of microsatellite loci and multiplex groups will allow time and cost savings. In this context, it can be said that panels containing six or more microsatellite markers can be used safely in paternity tests to be applied to Kilis goats. This application will be cheaper and more practical than other panels created with more microsatellite markers.

The calculated CPI values (<0.01) for all panels show that the microsatellites used have high individual identification power and are in the confidence interval indicated by Waits et al. (30). Obtained CPI values were in accordance with values reported by Luikart et al. (24).

The national animal breeding project is being carried out successfully by the General Directorate of Agricultural Research and Policy of the Ministry of Food, Agriculture, and Livestock in coordination with universities and breeders' associations. In this context, the habits of keeping records in small ruminants have been initiated and spread over time. On the other hand, with the numbering of the animals and breeding records, the possibility of obtaining the production potential information of the herds was ensured safely. Besides this possibility, it is important to note that special efficiency records can be kept and the efficiency records can be made continuous on the basis of the pedigree. However, the issue of controlling the correctness of reported outcomes and the problem of correct identification of parents is still being updated. In this context, it has been revealed that hand-mating in this study was recorded in field conditions with high accuracy.

It has also been found that it is possible to prove the validity of the obtained mating results using DNA-based microsatellite markers. On the other hand, the current study has developed cheap, fast, and reliable paternity test panels that can be used for Kilis goat populations. It can be stated that panels with a CPE above 0.999 can be used at the most reasonable cost and with high reliability in paternity tests to be performed in Kilis goats, which can be a reference for other populations. In order to provide more effective and widespread use of these panels, it is possible to test these panels in different breeds in the future and develop easy-to-use kits for them. This can contribute to the solution of the problem about verification of hand-

mating in large-area projects under breeder conditions such as the National Sheep and Goat Breeding Project.

Acknowledgments

The authors thank the Research Foundation of Mustafa Kemal University for its financial supports (Project No: 15422). We acknowledge the Republic of Turkey Ministry of Food, Agriculture, and Livestock for supplying animal materials and the Agricultural Biotechnology and Food Safety Application and Research Center (ADÖ-TARBIYOMER) of Adnan Menderes University for providing laboratory facilities to carry out the molecular genetic analysis.

References

1. Baron EE, Martinez ML, Vernequez RS, Coutinho LL. Parentage testing and effect of misidentification on the estimation of breeding value in Gir cattle. *Genet Mol Biol* 2002; 25: 389-394.
2. Taylor GR. *Laboratory Methods for the Detection of Mutations and Polymorphisms in DNA*. Boca Raton, FL, USA: CRC Press; 1997.
3. Parlato E, Van Vleck LD. Effect of parentage misidentification on estimates of genetic parameters for milk yield in the Mediterranean Italian buffalo population. *J Dairy Sci* 2012; 95: 4059-4064.
4. Weller JI, Feldmesser E, Golik M, Tager CI, Domochofsky R, Alus O, Ezra E, Ron M. Factors affecting incorrect paternity assignment in the Israeli Holstein population. *J Dairy Sci* 2004; 87: 2627-2640.
5. Yılmaz O, Karaca O. Paternity analysis with microsatellite markers in Karya sheep. *Kafkas Univ Vet Fac* 2012; 18: 807-813.
6. Yılmaz O. Power of different microsatellite panels for paternity analysis in sheep. *Anim Sci Pap Rep* 2016; 34: 155-164.
7. Bulut Z, Kurar E, Özşensoy Y, Altınok V, Nizamoglu M. Usefulness of microsatellite DNA markers for parentage testing for some goat population in Turkey. *J Cell Molbiol* 2014; 12: 39-45.
8. Karaca O, Yılmaz O, Ata N, Sevim S, Cemal I. Paternity testing for verification of pedigree information obtained from hand-mated Hair goats. In: *Proceedings of International Agricultural Science Congress*; Van, Turkey; 2018. p. 147.
9. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; 16: 1215.
10. Montgomery GW, Sise JA. Extraction of DNA from sheep white blood cells. *New Zeal J Agr Res* 1990; 33: 437-441.
11. FAO. *Molecular Genetic Characterization of Animal Genetic Resources*. Rome, Italy: Food and Agricultural Organization of the United Nations; 2011.
12. Hecker KH, Roux KH. High and low annealing temperatures increase both specificity and yield in touchdown and stepdown PCR. *Biotechniques* 1996; 20: 478-485.
13. Peakall R, Smouse PE. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics* 2012; 28: 2537-2539.
14. Marshall TC, Slate J, Kruuk LEB, Pemberton JM. Statistical confidence for likelihood-based paternity inference in natural populations. *Mol Ecol* 1998; 7: 639-655.
15. Brenner C, Morris CJ. *Paternity Index Calculations in Single Locus Hypervariable DNA Probes: Validation and Other Studies*. PowerStatsV12.xls Computer Software; 1990.
16. Paetkau D, Strobeck C. The molecular basis and evolutionary history of a microsatellite null allele in bears. *Mol Ecol* 1995; 4: 519-520.
17. Bhat RA, Uddin Reshi M, Beigh SA, Ahad WA, Padder TA, Ganai NA, Andrabi M, Shah RA. Microsatellite marker based DNA fingerprinting for parentage verification in goat breeds of Kashmir. *Int J Curr Microbiol App Sci* 2017; 6: 1131-1134.
18. Da Silva EC, McManus CM, Guimaraes MP, Gouveia AM, Facó O, Pimentel DM, Caetano AR, Paiva SR. Validation of a microsatellite panel for parentage testing of locally adapted and commercial goats in Brazil. *Genet Mol Biol* 2014; 37: 54-60.
19. Tefiel H, Ata N, Chahbar M, Benyarou M, Fantezi K, Yılmaz O, Cemal I, Karaca O, Boudouma D, Gaouar SBS. Genetic characterization of four Algerian goat breeds assessed by microsatellite markers. *Small Rumin Res* 2018; 160: 65-71.
20. Garritsen C, Van Marle-Köster E, Snyman MA, Visser C. The impact of DNA parentage verification on breeding value estimation and sire ranking in South African Angora goats. *Small Rumin Res* 2015; 124: 30-37.
21. Siwek M, Knol EF. Parental reconstruction in rural goat population with microsatellite markers. *Ital J Anim Sci* 2010; 9: 260-264.

22. Bolormaa S, Ruvinsky A, Walkden Brown S, Van der Werf J. DNA-based parentage verification in two Australian goat herds. *Small Rumin Res* 2008; 80: 95-100.
23. Dakin EE, Avise JC. Microsatellite null alleles in parentage analysis. *Heredity* 2004; 93: 504-509.
24. Luikart G, Biju-Duval MP, Ertuğrul O, Zagdsuren Y, Maudet C, Taberlet P. Power of 22 microsatellite markers in fluorescent multiplexes for parentage testing in goats (*Capra hircus*). *Anim Genet* 1999; 30: 431-438.
25. Van Eenennaam AL, Weaber RL, Drake DJ, Penedo MCT, Quaas RL, Garrick DJ, Pollak EJ. DNA-based paternity analysis and genetic evaluation in a large, commercial cattle ranch setting. *J Anim Sci* 2007; 85: 3159-3169.
26. ISAG. Applied genetics in sheep and goats workshop. In: Proceedings of 32nd International Conference on Animal Genetics; Edinburgh, UK; 2010.
27. Ganai NA, Yadav BR. Parentage determination in three breeds of Indian goat using heterologous microsatellite markers. In: Makkar HPS, Viljoen GJ, editors. Applications of Gene-Based Technologies for Improving Animal Production and Health in Developing Countries. Berlin, Germany: Springer; 2005. pp. 613-620.
28. Glowatzki-Mullis ML, Muntwyler J, Gaillard C. Cost-effective parentage verification with 17-plex PCR for goats and 19-plex PCR for sheep. *Anim Genet* 2007; 38: 86-88.
29. MAPA. White Paper. Brasilia, Brazil: Brazilian Ministry of Agriculture Livestock and Food Supply; 2004.
30. Waits LP, Luikart G, Taberlet P. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Mol Ecol* 2001; 10: 249-256.