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RESEARCH ARTICLE

Genetic Characterization of Turkish Cattle Breeds by Microsatellite Markers: Usefulness for Parentage Testing [1]

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Summary

Objective of this study was to evaluate microsatellite markers in paternity testing of native cattle breeds in Turkey. Blood samples were collected from Anatolian Black (n=51), Anatolian Grey (n=54), South Anatolian Red (n=51), Native Southern Anatolian Yellow (n=51), East Anatolian Red (n=45) and Zavot (n = 19) cattle. From the blood samples DNA was isolated by using a standard phenol/chloroform method. A total of 20 microsatellite loci were selected from a FAO/ISAG-suggested list. Polymerase chain reaction products were separated by capillary electrophoresis and marker genotypes were determined by fragment analysis. In statistical analyses, allel numbers, observed (Ho) and expected (He) heterozygosities, deviation from Hardy-Weinberg Equilibrium and probability of exclusion (PE) at each microsatellite locus were calculated. A total of 269 different alleles were observed and the mean allele was identified as 13.45. Mean Ho and He values were observed as 0.619-0.852 and 0.669-0.877, respectively. The results indicated that the microsatellite test panel including the most informative 7 loci had total PE value of >0.9999 in each populations and can thereby be used for parentage testing studies of native cattle breeds in Turkey.

Keywords: *Cattle, Microsatellite, Parentage testing, TURKHAYGEN-I*

Türkiye Yerli Sığır Irklarının Mikrosatellit Belirteçler ile Genetik Karakterizasyonu: Kimliklendirme Çalışmalarında Kullanılabilirliği

Özet

Bu çalışmanın amacı, mikrosatellit belirteçlerinin Türkiye yerli sığır ırklarının kimliklendirme çalışmalarında kullanılabilirliğinin araştırılmasıdır. Yerli Kara (n=51), Boz Irk (n=54), Güney Anadolu Kırmızısı (n=51), Yerli Güney Sarısı (n=51), Doğu Anadolu Kırmızısı (n=45) ve Zavot (n=19) ırkı sığırlardan alınan kan örneklerinden standart fenol/kloroform yöntemi ile DNA izolasyonu yapılmıştır. Çalışmada kullanılan 20 mikrosatellit lokusu FAO/ISAG tarafından tavsiye edilen listeden seçilmiştir. Yükseltgenen Polimeraz Zincir Reaksiyonu ürünleri kapiller elektroforez ile ayrıştırılmış ve fragman analizi ile lokus genotipleri tespit edilmiştir. İstatistiksel analizlerde, toplam allel sayısı, gözlenen (Ho) ve beklenen (He) heterezigotluk, Hardy-Weinberg Dengesine uygunluk ve dışlama gücü olasılığı parametreleri hesaplanmıştır. Toplam 269 allel gözlenmiş ve ortalama allel sayısı 13.45 olarak tespit edilmiştir. Ortalama Ho ve He değerleri sırasıyla 0.619-0.852 ve 0.669-0.877 tespit edilmiştir. Enformatif 7 lokusu içeren mikrosatellit panelinin toplam dışlama gücü olasılığının tüm ırklarda >0.9999 olacağı ve yerli sığır ırklarının kimliklendirme çalışmalarında başarıyla kullanılabileceği tespit edilmiştir.

Anahtar sözcükler: *Sığır, Mikrosatellit, Kimliklendirme, TÜRKHAYGEN-I*

INTRODUCTION

Paternity testing is widely used in criminal cases, biomedical researches, and in cases of determination of inbreeding levels in different population. While protein polymorphism, blood antigens, and tissue proteins were

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previously used for this purpose, DNA-based different test panels (RFLP, AFLP, RAPD, mtDNA etc.) were developed and found to be more efficient. Comparing with other marker systems, microsatellites [1], exist widely in the genome, have features of higher polymorphism and codominant inheritance [2]. Due to their high polymorphism and technical ease including suitability for PCR technology and capillary electrophoresis, microsatellites are widely preferred in the paternity testing efforts of various mammalian species [3]. Correct determination of genetic relationships among animal populations has critical importance for development of selection programs [3], generation of pedigree structures [4], estimation of heritability [5,6], and breeding values [7]. Significantly higher rates of paternity misidentification were reported even in the countries where herd records are performed with great care [3,5,8].

Significant levels of incorrect paternity (2.90-15%) were reported in analyses made with marker systems [5,9-11]. Moreover, misidentification rates were reported to be higher in females [9,11]. Paternity misidentification was determined to cause serious deviations (5-50%) in the estimation of genetic parameters and reduction of genetic gain in selection programs [12]. It was reported that more than 20% of paternity misidentification cases were caused by artificial insemination of more than one bull [3,11] and it can be reduced to 8% by using a quality control system which results in an increase of 1% in genetic progress [11]. It is well known that herd improvement can be performed to have extra profits by accurate estimation of parent identification [9]. In addition, even though the possibility of paternity identification for the estimation of breeding value is desired, there is still need for more cost-effective tests in commercial mean [7].

Modern dairy and beef industry focus on using a number of highly productive cattle breeds. On the other hand, local breeds are often accepted as uneconimal and certain biotechnologycal applications can not be used because of costliness. There is an increasing demand for paternity testing in breeding programs of native animal breeds. Due to population properties, special paternity testing panels are needed for some native cattle breeds in which certain loci can be uninformative. Also, an informative test panel including the lowest possible number of the most informative loci can offer economical and pratical paternity testing possibilities. Objective of this study was the evaluation of microsatellite markers in paternity testing in native cattle breeds in Turkey as part of a national project titled "In vitro Conservation and Preliminary Molecular Identification of Some Turkish Domestic Animal Genetic Resources-1 (TURKHAYGEN-I)".

MATERIAL and METHODS

A total of 271 blood samples were collected from South Anatolian Red (SAR, n = 51), Native Southern Anatolian Yellow (SAY, n = 51), Anatolian Black (AB, n = 51), Anatolian Grey (AG, n = 54), East Anatolian Red (EAR, n = 45) and Zavot (ZAV, n = 19) cattle. Genomic DNA samples were extracted by using a standard organic phenol/chloroform method [13]. A total of 20 microsatelllite loci *(Table 1)* were selected from a list [14] suggested by FAO MoDAD and International Society of Animal Genetics (ISAG).

Microsatellite genotyping procedures were described elsewhere [15]. Briefly each multiplex PCR was performed in 15 µl reaction volume including 1x Mg^{++} free PCR buffer (Fermentas), 0.125 mM dNTPs (Fermentas), 1.5 mM $MgCl^{++}$, 0.375 U of *Tag* polymerase (Fermentas), 2 - 17 pMol each primer and ~100 ng of genomic DNA.

A touchdown-PCR profile [16] was used with two steps. The first step was initial denaturation at 95°C for 4 min, followed by 16 cycles of denaturation at 94°C for 30 sec, annealing beginning at 60°C and ending at 52°C for 30 sec and extension at 72°C for 30 sec. The annealing temperature was decreased 0.5°C per cycle until it reached 52°C. At the second step, 25 cycles of 94°C for 30 sec, 52°C for 30 sec and 72°C for 30 sec was applied. The final extension of 72°C for 10 min was applied in all reactions. The resulting PCR products were denaturated in Hi-Di-formamide including S-400 DNA size standart and loaded onto a Beckman Coulter CEQ-8000 Genetic Analysis System for capillary electrophoresis. Genotypes were determined by fragment analysis using CEQ-8000 FragTest program. General population parameters including allele number (Na), expected (He) and observed (Ho) heterozigosities, deviation from Hardy-Weinberg Equilibrium (HWE) and probability of exclusion for each locus (PE-1=Both parents known and PE-2=Only one parent known) were calculated using GenAlEx6 [17] package program.

RESULTS

In this study, 20 microsatellite loci were separated by capillary electrophoresis and allele genotypes in each marker locus were determined by fragment analysis. Three different multiplex pool systems were formed including 7 (CSSM66, ETH03, HEL9, CSRM60, INRA023, SPS115, ILSTS006), 7 (INRA005, HAUT27, TGLA122, TGLA126, TGLA227, BM1824, HEL13) and 6 (BM2113, TGLA53, ETH225, ETH10, ETH185, BM1818) loci.

Observed allele numbers (Na), expected (He) and observed (Ho) heterozygoties deviations from Hardy-Weinberg Equilibrium (HWE) were summarized in *Table 2* and *3*. In this study, a total of 269 different alleles were detected. The mean allele number was 13.45. The maximum and minimum numbers of total alleles were observed in TGLA122 (26 alleles) and INRA005 (7 alleles), respectively. The highest average observed (Ho) and expected (He) heterozygosity values were determined as 0.619-0.852 and 0.669-0.877, respectively. HWE's were

Table 1. *Microsatellite loci used in the study*
Tablo 1. *Kullanılan mikrosatellit markör listesi*

No	Locus	Chromosome	Primer	Allele
1	BM1824	1	GAGCAAGGTGTTTTTCCAATC	170-218
			CATTCTCCAACTGCTTCCTTG	
2	BM2113	2	GCTGCCTTCTACCAAATACCC	116-146
			CTTAGACAACAGGGGTTTGG	
3	INRA023	3	GAGTAGAGCTACAAGATAAACTTC	193-235
			TAACTACAGGGTGTTAGATGAACTCA	
4	ETH10	5	GTTCAGGACTGGCCCTGCTAACA	198-234
			CCTCCAGCCCACTTTCTCTTCTC	
5	ILSTS006	7	TGTCTGTATTTCTGCTGTGG	277-309
			ACACGGAAGCGATCTAAACG	
6	HEL9	8	CCCATTCAGTCTTCAGAGGT	141-173
			CACATCCATGTTCTCACCAC	
7	ETH225	9	GATCACCTTGCCACTATTTCCT	135-165
			ACATGACAGCCAGCTGCTACT	
8	CSRM60	10	AAGATGTGATCCAAGAGAGAGGCA	79-115
			AGGACCAGATCGTGAAAGGCATAG	
9	HEL13	11	TAAGGACTTGAGATAAGGAG	178-200
			CCATCTACCTCCATCTTAAC	
10	INRA005	12	CAATCTGCATGAAGTATAAATAT	135-149
			CTTCAGGCATACCCTACACC	
11	CSSM66	14	ACACAAATCCTTTCTGCCAGCTGA	171-209
			AATTTAATGCACTGAGGAGCTTGG	
12	SPS115	15	AAAGTGACACAACAGCTTCTCCAG	235-265
			AACGAGTGTCCTAGTTTGGCTGTG	
13	TGLA53	16	GCTTTCAGAAATAGTTTGCATTCA	143-191
			ATCTTCACATGATATTACAGCAGA	
14	ETH185	17	TGCATGGACAGAGCAGCCTGGC	214-246
			GCACCCCAACGAAAGCTCCCAG	
15	TGLA227	18	CGAATTCCAAATCTGTTAATTTGCT	64-115
			ACAGACAGAAACTCAATGAAAGCA	
16	ETH03	19	GAACCTGCCTCTCCTGCATTGG	90-135
			ACTCTGCCTGTGGCCAAGTAGG	
17	TGLA126	20	CTAATTTAGAATGAGAGAGGCTTCT	104-131
			TTGGTCTCTATTCTCTGAATATTCC	
18	TGLA122	21	CCCTCCTCCAGGTAAATCAGC	134-193
			AATCACATGGCAAATAAGTACATAC	
19	BM1818	23	AGCTGGGAATATAACCAAAGG	248-278
			AGTGCTTTCAAGGTCCATGC	
20	HAUT27	26	TTTTATGTTCATTTTTTGACTGG	120-158
			AACTGCTGAAATCTCCATCTTA	

found to be insignificant mostly in ZAV (17 loci) and at least in AG (10 loci). Some loci were significantly deviated from HWE.

Power of exclusion values were calculated in the presence of one parent (PE-2) and two parents (PE-1) *(Table 4)*. PE-2 values varied between 0.328 (INRA005, TGLA126 and BM1824) and 0.806 (TGLA122). The lowest PE-1 value (0.504) was observed in SAY and ZAV populations in INRA005, TGLA126 and BM1824, the highest PE-1 value (0.893) was determined in SAR and AB populations for TGLA122 locus. The highest and the lowest average

Table 2. *The number of alleles*
Tablo 2. *Populasyonlarda gözlenen allel sayıları*

Locus	Populations						Mean	Total
	SAR	AB	AG	SAY	EAR	ZAV		
CSSM66	13	13	12	13	13	9	12.17	14
CSRM60	10	13	11	12	7	6	9.83	15
ETH03	10	11	10	11	10	11	10.50	14
INRA023	13	10	10	11	10	9	10.50	14
HEL9	11	12	12	14	11	10	11.67	16
ILSTS006	11	11	10	9	8	5	9.00	13
SPS115	9	10	8	9	8	6	8.33	10
ETH185	12	12	12	13	10	9	11.33	17
BM1818	8	10	10	11	8	7	9.00	13
ETH225	13	11	8	9	10	10	10.17	13
ETH10	8	8	8	9	7	5	7.50	9
TGLA53	18	14	18	19	11	13	15.50	23
BM2113	10	9	9	12	8	9	9.50	13
INRA005	5	6	6	4	6	4	5.17	7
HAUT27	8	9	9	8	9	7	8.33	10
TGLA122	19	19	15	17	16	12	16.33	26
TGLA126	6	8	8	9	8	4	7.17	9
TGLA227	12	12	13	13	11	11	12.00	16
BM1824	6	7	5	5	5	4	5.33	8
HEL13	8	7	7	8	6	5	6.83	9
Mean	10.50	10.60	10.05	10.80	9.10	7.80	9.81	13.45

Table 3. *Observed (Ho) and expected (He) heterozygosity and Hardy-Weinberg Equilibrium (HWE)*
Tablo 3. *Gözlenen (Ho) ve beklenen (He) heterozigotluk ile Hardy-Weinberg Dengesi (HWE)*

Locus	Mean		HWE					
	Ho	He	SAR	AB	AG	SAY	EAR	ZAV
CSSM66	0.822	0.856	ns	ns	***	ns	ns	ns
CSRM60	0.761	0.762	ns	ns	**	ns	ns	ns
ETH3	0.762	0.804	ns	ns	ns	ns	ns	ns
INRA023	0.779	0.808	ns	ns	ns	ns	***	ns
HEL9	0.793	0.834	ns	ns	ns	*	ns	ns
ILSTS006	0.673	0.755	*	ns	ns	***	ns	ns
SPS115	0.661	0.768	**	*	***	*	ns	**
ETH185	0.797	0.788	ns	ns	**	*	***	***
BM1818	0.767	0.771	ns	ns	***	ns	***	ns
ETH225	0.742	0.814	***	***	***	ns	**	ns
ETH10	0.644	0.669	***	ns	ns	*	ns	ns
TGLA53	0.801	0.877	**	**	**	**	ns	ns
BMS2113	0.806	0.840	*	ns	***	ns	ns	ns
INRA005	0.671	0.685	ns	ns	ns	ns	ns	ns
HAUT27	0.619	0.734	ns	*	***	***	***	*
TGLA122	0.794	0.842	ns	**	ns	ns	*	ns
TGLA126	0.750	0.759	ns	ns	ns	ns	ns	ns
TGLA227	0.852	0.859	ns	ns	*	**	ns	ns
BM1824	0.719	0.711	ns	ns	ns	ns	ns	ns
HEL13	0.728	0.788	ns	ns	ns	ns	ns	ns

Ho: *Observed,* ***He:*** *Expected Heterozygosity,* ***HWE:*** *Hardy-Weinberg Equilibrium,* ***ns*** *= non significant, * P<0.05, ** P<0.01, *** P<0.001*

Table 4. *Probability of Exclusion for each locus (PE-1=both parents known and PE-2= only one parent known)*
Tablo 4. *Dışlama Gücünün 1 (DG-1) ve 2 (DG-2) ebeveyn varlığında değerleri*

Locus	Populations											
	SAR		AB		AG		SAY		EAR		ZAV	
	PE-2	PE-1	PE-2	PE-1	PE-2	PE-1	PE-2	PE-1	PE-2	PE-1	PE-2	PE-1
CSSM66	0.726	0.842	0.726	0.842	0.707	0.829	0.726	0.842	0.726	0.842	0.626	0.772
CSRM60	0.657	0.795	0.726	0.842	0.684	0.813	0.707	0.829	0.542	0.707	0.486	0.660
ETH03	0.657	0.795	0.684	0.813	0.657	0.795	0.684	0.813	0.657	0.795	0.684	0.813
INRA023	0.726	0.842	0.657	0.795	0.657	0.795	0.684	0.813	0.657	0.795	0.626	0.772
HEL9	0.684	0.813	0.707	0.829	0.707	0.829	0.744	0.854	0.684	0.813	0.657	0.795
ILSTS006	0.684	0.813	0.684	0.813	0.657	0.795	0.626	0.772	0.588	0.743	0.416	0.595
SPS115	0.626	0.772	0.657	0.795	0.588	0.743	0.626	0.772	0.588	0.743	0.486	0.660
ETH185	0.707	0.829	0.707	0.829	0.707	0.829	0.726	0.842	0.657	0.795	0.626	0.772
BM1818	0.588	0.743	0.657	0.795	0.657	0.795	0.684	0.813	0.588	0.743	0.542	0.707
ETH225	0.726	0.842	0.684	0.813	0.588	0.743	0.626	0.772	0.657	0.795	0.657	0.795
ETH10	0.588	0.743	0.588	0.743	0.588	0.743	0.626	0.772	0.542	0.707	0.416	0.595
TGLA53	0.796	0.887	0.744	0.854	0.796	0.887	0.806	0.893	0.684	0.813	0.726	0.842
BM2113	0.657	0.795	0.626	0.772	0.626	0.772	0.707	0.829	0.588	0.743	0.626	0.772
INRA005	0.416	0.595	0.486	0.660	0.486	0.660	0.328	0.504	0.486	0.660	0.328	0.504
HAUT27	0.588	0.743	0.626	0.772	0.626	0.772	0.588	0.743	0.626	0.772	0.542	0.707
TGLA122	0.806	0.893	0.806	0.893	0.759	0.864	0.785	0.880	0.773	0.872	0.707	0.829
TGLA126	0.486	0.660	0.588	0.743	0.588	0.743	0.626	0.772	0.588	0.743	0.328	0.504
TGLA227	0.707	0.829	0.707	0.829	0.726	0.842	0.726	0.842	0.684	0.813	0.684	0.813
BM1824	0.486	0.660	0.542	0.707	0.416	0.595	0.416	0.595	0.416	0.595	0.328	0.504
HEL13	0.588	0.743	0.542	0.707	0.542	0.707	0.588	0.743	0.486	0.660	0.416	0.595
Mean	0.645	0.782	0.657	0.792	0.638	0.778	0.651	0.785	0.611	0.758	0.545	0.700
Total	>0.9999		>0.9999		>0.9999		>0.9999		>0.9999		>0.9999	

PE-2 and PE-1 values were determined in ZAV and AB populations. Total PE-2 and PE-1 values were calculated as >0.999 for all populations using the most polymorphic 7 (CSSM66 + CSRM60 + ETH03 + INRA023 + HEL9 + ILSTS006 + SPS115) and 5 (CSSM66 + CSRM60 + ETH03 + INRA023 + HEL9) loci, respectively.

DISCUSSION

Of 20 microsatellites used in this study, 12 loci were reported as the most commonly used for cattle parentage testing [18] and all loci were highly polymorphic. Observed high polymorphisms suggest that these loci are appropriate to be used in population genetic studies. Obtained average allele number (13.45) was found to be similar with the other studies of Turkish native cattle breeds [19-21]. The highest allele number at TGLA122 was also observed in previous studies [21-24].

Informativeness of a locus depends on the allele number. For this purpose, the parameters including heterozygosity (Ho) and probability of exclusion values were widely used and estimated by distribution of allele frequencies in populations. The Ho and He values of native cattle breeds in Turkey were determined to be higher than that reported for other breeds from different continents [19,24-26]. The reason for the higher Na, Ho and He is thought to be the number of samples used, the close localization of these populations to the domestication region and high level of genetic diversity [15,19,20,27].

Probability of exclusion (PE) is a mathematical definition of probability of excluding a random individual from the population as a potential parent. The PE is accepted as the most important criteria for genetic markers used in parentage testing studies [28]. In the present study, adequate PE values (>0.999) were observed for all cattle populations using 20 markers.

Different population genetic parameters and the misidentification rate were investigated by using 9 different microsatellite markers for Gry cows located in Brazil [29]. By using the same 7 [29] and 11 markers [3] in this study, PE values have been found to be 0.188-0.629 [29] and 0.175-0.552 [3] for Gry and Yugoslav Pied cattle, respectively. The total PE values were 0.979 [29] and 0.996 [3]. The total PE values were estimated for Holstein-Friesian, Brown Swiss

and their crosses with native cattle breeds in Turkey [30] and found to be similar (>0.9999) with this study.

The PE >0.999 was obtained with 9 [31] and 10 markers [4], however, the same PE was found in this study by only using 7 (PE-2) and 5 markers (PE-1). Recently SNPs were reported to be efficient marker system parentage testing efforts [32].

Basen on the results of this study; it was concluded that a test panel including the most informative 7 loci can provide enough power proving its usefulness for parentage testing and population genetic studies of local cattle breeds in Turkey.

REFERENCES

1. Weber JL, May PE: Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am J Hum Genet,* 44, 388-396, 1989.

2. Özşensoy Y, Kurar E: Markör sistemleri ve genetik karakterizasyon çalışmalarında kullanımları. *J Cell Mol Biol,* 10 (2): 11-19, 2012.

3. Stevanovic J, Stanimirovic Z, Dimitrijevic V, Maletic M: Evaluation of 11 microsatellite loci for their use in paternity testing in Yugoslav Pied cattle (YU Simmental cattle). *Czech J Anim Sci,* 55 (6): 221-226, 2010.

4. Carolino I, Sousa CO, Ferreira S, Carolino N, Silva FS, Gama LT: Implementation of a parentage control system in Portuguese beef-cattle with a panel of microsatellite markers. *Genet Mol Biol,* 32 (2): 306-311, 2009.

5. Geldermann H, Pieper U, Weber WE: Effect of misidentification on the estimation of breeding value and heritability in cattle. *J Anim Sci*, 63, 1759-1768, 1986.

6. Dechow CD, Norman HD, Zwald NR, Cowan CM, Meland OM: Relationship between individual herd-heritability estimates and sire misidentification rate. *J Dairy Sci,* 91 (4): 1640-1647, 2008.

7. Banos G, Wiggans GR, Powell RL: Impact of paternity errors in cow identification on genetic evaluations and international comparisons. *J Dairy Sci,* 84 (11): 2523-2529, 2001.

8. Ozkan E, Soysal MI, Ozder M, Koban E, Sahin O, Togan I: Evaluation of parentage testing in the Turkish Holstein population based on 12 microsatellite loci. *Livestock Sci,* 124, 101-106, 2009.

9. Ron M, Blanc Y, Band M, Ezra E, Weller JI: Misidentification rate in the Israeli dairy cattle population and its implications for genetic improvement. *J Dairy Sci,* 79 (4): 676-681, 1996.

10. Ron M, Domochovsky R, Golik M, Seroussi E, Ezra E, Shturman C, Weller JI: Analysis of vaginal swabs for paternity testing and Marker-Assisted Selection in cattle. *J Dairy Sci,* 86 (5): 1818-1820, 2003.

11. Weller JI, Feldmesser E, Golik M, Tager-Cohen I, Domochovsky R, Alus O, Ezra E, Ron M: Factors affecting incorrect paternity assignment in the Israeli Holstein population. *J Dairy Sci,* 87 (8): 2627-2640, 2004.

12. Senneke SL, MacNeil MD, Van Vleck LD: Effects of sire misidentification on estimates of genetic parameters for birth and weaning weights in Hereford cattle. *J Anim Sci,* 82 (8): 2307-2312, 2004.

13. Sambrook J, Fritsch EF, Maniatis T: Molecular Clonning: A Laboratory Manual. 2nd ed., 9.16-9.19, Cold-Spring Harbor, New York, USA, 1989.

14. Hoffmann I, Marsan PA, Barker JSF, Cothran EG, Hanotte O, Lenstra JA, Milan D, Weigend S, Simianer H: New MoDAD marker sets to be used in diversity studies for the major farm animal species: Recommendations of a joint ISAG/FAO working group. **In,** *Proceedings of the 29th ISAG (International Society of Animal Genetics) Congress*. 11-16 September, Tokyo, 2004.

15. Özşensoy Y, Kurar E, Doğan M, Bulut Z, Altunok V, Işık A, Çamlıdağ A, Nizamlıoğlu M: Türkiye'de bulunan bazı yerli sığır ırklarının STR markörler ile genetik karakterizasyonu. *BİBAD,* 3, 163-171, 2010.

16. Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS: Touchdown PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Res,* 19, 4008, 1991.

17. Peakall R, Smouse PE: GENALEX 6: Genetic analysis in excel. Population genetic software for teaching and research. *Mol Ecol Notes,* 6, 288-295, 2006.

18. ISAG Conference: Edinburg, Scotland. Cattle molecular marker and parentage testing workshop, 2010. http://www.isag.us/Docs/CattleMMPTest_CT.pdf, *Accessed:* 01.03.2013.

19. Loftus RT, Ertuğrul O, Harba MH, El-Barody AA, MacHugh DE, Park SDE, Bradly DG: A microsatellite survey of cattle from a centre of origin: The Near East. *Mol Ecol,* 8 (12): 2015-2022, 1999.

20. Cymbron T, Freeman AR, Isabel Malheiro M, Vigne JD, Bradley DG: Microsatellite diversity suggests different histories for Mediterranean and Northern European cattle populations. *Proc Biol Sci,* 272 (1574): 1837-1843, 2005.

21. Özkan E: Türkiye'de yetiştirilen yerli ve kültür sığır ırklarının genetik yapılarının mikrosatelitler ile incelenmesi. *Doktora Tezi,* Trakya Üniv. Fen Bil. Enst., 2005.

22. Kim KS, Yeo JS, Choi CB: Genetic diversity of North-East Asian cattle based on microsatellite data. *Anim Genet,* 33, 201-204, 2002.

23. Maudet C, Luikart G, Taberlet P: Genetic diversity and assignment tests among seven French cattle breeds based on microsatellite DNA analysis. *J Anim Sci,* 80, 942-950, 2002.

24. Mao Y, Chang H, Yang Z, Zhang L, Xu M, Chang G, Sun W, Song G, Ji D: The analysis of genetic diversity and differentiation of six Chinese cattle populations using microsatellite markers. *J Genet Genomics,* 35, 25-32, 2008.

25. MacHugh DE, Shriver MD, Loftus RT, Cunningham P, Bradley DG: Microsatellite DNA variation and the evolution, domestication and phylogeography of taurine and zebu cattle *(Bos taurus* and *Bos indicus)*. *Genetics,* 146 (3): 1071-1086, 1997.

26. Cañón J, Alexandrino P, Bessa I, Carleos C, Carretero Y, Dunner S, Ferran N, Garcia D, Jordana J, Laloë D, Pereira A, Sanchez A, Moazami-Goudarzi K: Genetic diversity measures of local European beef cattle breeds for conservation purposes. *Genet Sel Evol,* 33 (3): 311-332, 2001.

27. Troy CS, MacHugh DE, Bailey JF, Magee DA, Loftus RT, Cunningham P, Chamberlain AT, Sykes BC, Bradley DG: Genetic evidence for Near-Eastern origins of European cattle. *Nature,* 410 (6832): 1088-1091, 2001.

28. Cifuentes LO, Martinez EH, Acuna MP, Jonquera HG: Probability of exclusion in paternity testing: Time to reassess. *J Forensic Sci,* 51 (2): 349-350, 2006.

29. Curi RA, Lopes CR: Evaluation of nine microsatellite loci and misidentification paternity frequency in a population of Gyr breed bovines. *Braz J Vet Res Anim Sci*, 39 (3): 129-135, 2002.

30. Kurar E, Bulut Z, Nizamlıoğlu M: Sığır mikrosatellit test panelinin Türkiye'de ebeveyn tayini çalışmalarında kullanılabilirliği. *Eurasian J Vet Sci,* 29 (1): 24-29, 2013.

31. Tian F, Sun D, Zhang Y: Establishment of paternity testing system using microsatellite markers in Chinese Holstein. *J Genet Genomics,* 35, 279-284, 2008.

32. Hara K, Kon Y, Sasazaki S, Mukai F, Mannen H: Development of novel SNP system for individual and pedigree control in a Japanese Black Cattle population using whole-genome genotyping assay. *Anim Sci J,* 81 (4): 506-512, 2010.