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PARENTAGE TESTING IN DIFFERENT BREEDS OF DOGS USING MICROSATELLITE MARKERS

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ABSTRACT

A total of 45 blood samples from progeny and the putative parents were tested for parentage analysis using a panel of microsatellite markers (short tandem repeats). Six breeds of dogs were represented viz. German shepherd, Dalmatian, Labrador, Great Dane, Boxer and Lhasa Apso in this study for genotype analysis. Ten different microsatellite marker loci were amplified by multiplex PCR. The multiplex PCR products were run in genetic analyzer. The data were analyzed using gene mapper software to measure the allele size. Such measured allele size was compared to determine whether there are matches between the progeny and the putative parents. In conclusion, short tandem repeats uniformly distributed in the genome was found to be highly polymorphic and can be used as molecular tool for parentage testing in dogs of different breeds.

Key words*:* microsatellite markers, dog parentage, multiplex PCR..

INTRODUCTION

Genotyping is an emerging molecular tool to establish the parentage of individuals which becomes important to analyze the intra-specific variations that are helpful in knowing about the breeds. Jeffreys (1985) introduced the DNA finger printing method based on the simultaneous identification of several highly polymorphic repetitive sequences (mini satellites) in the genome. This technique was further developed by Nakamura (1987) for the use of locus specific probes for the detection of minisatellites. Microsatellites are the most frequently used type of markers in linkage mapping. They consist of short repeats of one to six nucleotides reiterated 10-15 times, are evenly distributed, numerous, polymorphic and are present in most vertebrates according to Stallings (1991) and Hearne (1992).

Microsatellite marker panels developed for domesticated and highly selected animals are uniquely challenging for these purpose. The study was focused on how the highly polymorphic repetitive sequences (microsatellite) in the genome can be used as marker for parentage analysis of different breeds of dogs.

MATERIALS AND METHODS

Samples:

A total of 3ml of forty five EDTA stabilized blood was collected from pups and putative parents in Chennai, Tamil Nadu, India. The following six breeds were represented viz., German shepherd, Dalmatian, Labrador, Great Dane, Boxer and Lhasa apso in this study.

DNA isolation:

Genomic DNA was extracted using conventional phenol-chloroform method and the final DNA was resuspended in 100 µl of Tris-EDTA buffer, pH-8.0 and further used for multiplex PCR.

Multiplex PCR:

A multiple fluorescent dye based technology for multiplex PCR analysis was used in this study to detect the tandem repeat polymorphisms targeting the 10 canine microsatellite regions using specific already published markers given in the table 1 as described by Cho (2005). The multiplex PCR reaction mix and thermal cycling profile were followed as given below. Amplification was performed, using 2400 Gene Amp PCR system, in a total volume of 9 µL containing 1 µL of isolated DNA, 2.8 µL of 8 mol primer mix, 0.36 µL of 25 mM $MgCl_2$, 2.2 µL of 25 mM dNTP mix, 0.36 µL of AmpliTaq Gold polymerase (5U/µL), 1.4 µL of 10X s reaction buffer and 1.9 µL of deionized water. The thermal cycling profile consisted of an initial denaturation for 10 min. at 95 ºC, followed by 20 cycles of 95 ºC for 30 sec, 58 ºC for 30 sec, 72 ºC for 60 sec and then 15 cycles of 95 ºC for 30 sec, 56 ºC for 30 sec, 72 ºC for 60 sec and a final elongation of 72 ºC for 30 min.

Electrophoresis using genetic analyzer:

The PCR products were diluted with sterile triple distilled water in the ratio 1:50, 1;100 and 1:200. Then, 1 µL of this diluted product from different dilutions was mixed with 11.5 µL of Hi-Di Formamide and 0.5 µL of size standard (Genescan-350 ROX) to yield a total of 13 µL reaction mix. This mixture was subjected to a denaturing condition of 95 ºC for 3 min and immediately snap-cooled on ice and then loaded in the ABI PRISM 3130 Genetic Analyzer.

Analysis of multiplex PCR products using gene mapper software:

The size was analyzed by GeneScan Analysis (Version 3.0) and Genotyper (Version 2.1) software (Applied Biosystems USA). Initially, panel (group of markers) and markers (fragment size range (bp), dye color and repeat length) was created in the gene mapper programme. Based on initial analysis of samples, bin set was created for each possible allele associated with a marker. Then, each sample was analyzed according to the bin set. The genotype quality values (QV) were assessed and suppose if the quality value is low that indicates issue with size standard. Then, it is needed to check all size standard peaks are present and labeled correctly to improve the quality values. The software defines each allele according to PCR product size and also gave the allele frequencies for each locus.

RESULTS AND DISCUSSION

Parentage testing was performed for a total of 15 pups representing 6 different breeds namely German shepherd, Dalmatian, Labrador, Great Dane, Boxer and Lhasa Apso. Two of them (sample 2 and 5) were found to be matching and 13 pups were not matching with the respective suspected dam and sire according to the 10 microsatellite markers used for genotyping as

Table 1: Primers used for multiplex PCR amplification of tandem repeats

Locus	Dye	Primer sequence		Expected size
		Forward-labelled (5' – 3')	Reverse labelled(5' – 3')	
PEZ1	FAM	GGCTGTCACTTTTCCCTTTC	CACCACAATCTCTCTCATAAATAC	92-136
FH2054	FAM	GCCTTATTCATTGCAGTTAGGG	ATGCTGAGTTTTGAACTTTCCC	140-183
FHC2010	FAM	AAATGGAACAGTTGAGCATGC	CCCCTTACAGCTTCATTTTCC	210-260
PEZ5	JOE	GCTATCTTGTTTCCCACAGC	TCACTGTATACAACATTGTC	97-121
PEZ20	JOE	CCTAAATTAGAGGTCTAACC	TAAGCGGGAATGTGCTCCTC	170-201
PEZ12	JOE	GTAGATTAGATCTCAGGCAG	TAGGTCCTGGTAGGGTGTGG	250-320
PEZ3	ATTO550	CACTTCTCATACCCAGACTC	CAATATGTCAACTATACTTC	95-154
PEZ6	ATTO550	ATGAGCACTGGGTGTTATAC	ACACAATTGCATTGTCAAAC	164-214
PEZ8	ATTO550	TATCGACTTTATCACTGTGG	ATGGAGCCTCATGTCTCATC	222-260
FHC2079	ATTO550	CAGCCGAGCACATGGTTT	ATGATTCTGATATGCCCAGC	263-299

mentioned in the table 1 viz., PEZ1, PEZ3, PEZ5, PEZ6, PEZ8, PEZ12, PEZ20, FHC2010, FHC2054, FHC2079. The entire locus was amplified in all the six different breeds. The results of matched (sample 2) and unmatched genotype (sample 6) of both parents and progeny were presented in Table 1 and 2 respectively. The ten panel of microsatellite marker (short tandem repeat) were found to be polymorphic and inherited unaltered from one generation to the next. In this study also, these microsatellite markers were found to be highly useful for parentage analysis as reported by earlier researchers Muller (1999), Ostrander (1995) and Zaje (1994). While running the undiluted amplified multiplex PCR products in genetic analyzer gave high signal intensity, which was unsuitable for genotyping analysis. Hence, the amplified products were further diluted in 1:50, 1:100 and 1:200 dilutions. In this study, 1: 50 dilution gave proper signal and suitable for genotype analysis. The variety of samples including semen, hair and blood can be used for genotyping analysis. However, blood is rich source for DNA and suitable for genotype analysis. The number of allele peak depends on the whether the animal tested is homozygote(single) or heterozygote (double) and the 4 bp shutter peak was also observed left to the each allele peak and the results coincide with earlier report of Walsh (1996). Most of the allele for each locus falls within the expected size range and few alleles that falls out of its expected size range during analysis. This indicates that such alleles may be present at extremely low frequencies in the population. A total of 20 panels of markers were used for accurate parentage analysis according to Ichikawa (2001). However in this study, only 10 panels of markers were successfully used in the breeds of German shepherd, Dalmatian, Labrador, Great Dane, Boxer and Lhasa apso. Hence, short tandem repeats microsatellite markers can be used as molecular tool for parentage testing in dogs of different breads.

Table 2. Matched genotypes for parentage analysis

Locus	Dam	Sire	Progeny
PEZ1	al 1(119.27)	al 1(119.36)	al 1(119.19)
PEZ3	al1,3(119.58,130.68)	al1,3(119.56,130.71)	al 1,3(119.61,130.72)
PEZ5	al 1(103.21)	al 1(103.29)	al 1(103.08)
PEZ6	al 1,2(175.36,184.0)	al1,3(175.29,189.59)	al 1,3(175.34,189.56)
PEZ8	al1,2(229.45,241.69)	al 2(241.73)	al 2(241.36)
PEZ12	al 1(270.89)	al 1(270.97,274.46)	al 1(270.72)
PEZ20	al 1(176.36)	al 1(176.39)	al 1(176.27)
FHC2010	al1,2(227.51,240.52)	al 2(240.67)	al 2(240.17)
FHC2054	al1,2(151.23,168.17)	al 1(151.33)	al 1(151.13)
FHC2079	al 2(276.57)	al 2(276.79)	al 2(276.26)

The size of amplified product for each locus is given in bracket

Table 3. Unmatched genotypes for genotypes analysis

Locus	Dam	Sire	Progeny
PEZ1	al 2(115.18)	al 2(114.95)	al 1,3(111.05,118.99)
PEZ3	al 1,3(119.57,127.57)	al 1,3(119.46,127.47)	al 1,3(119.32,127.30)
PEZ5	al 3(111.23)	al 1,3(102.95,111.0)	al 1,2(102.86,106.89)
PEZ6	al 2,3(183.02,191.55)	al 1,3(171.45,191.48)	al 2,3(183.12,191.69)
PEZ8	al 2(232.66)	al 2(232.34)	al 1,2(228.1,233.02)
PEZ12	al 2,3(267.27,270.78)	al 3(270.47)	al 1,4(259.62,300.76)
PEZ20	al 1,2(172.47,176.27)	al 2,3(175.98,183.85)	al 1,2(172.21,176.11)
FHC2010	al 1,2(227.2,231.57)	al 2(231.01)	al 3,4(234.89,240.21)
FHC2054	al 1(160.0)	al 2(163.57)	al 2,3(163.61,167.86)
FHC2079	al 2(276.31)	al 1(271.9)	al 2,3(276.14,279.6)

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