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Beagle dogs parentage testing by using 22 ISAG microsatellite markers

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Abstract : The objective of the study was to establish routine parentage testing system in Beagle dogs using 22 ISAG (International Society for Animal Genetics) canine microsatellite markers (2005). Blood collections were obtained from a mother dog, 4 candidate father dogs and 3 offspring (n = 8). Genomic DNA samples were extracted from 8 Beagle dogs blood for PCR analysis. PCR products for the allele were analyzed by ABI 3130 DNA Sequencer and GeneScan (*Ver* 3.0) analysis and Genotyper (*Ver.* 2.1) software. The genetic relationship of mother and 3 offspring as well as one father dog among 4 candidate father dogs was confirmed by microsatellite allele analysis. The results of locus for amelogenin, which was designed for sexing, were matching with real gender among 8 Beagle dogs (female; 217/217 homozygosity, male; 179/217 heterozygosity). Twenty two ISAG microsatellite markers are useful the parentage test of Beagle dogs. In addition, amelogenin is an applicable marker to detecting real sex in dogs.

Key words : Beagle, Microsatellite markers, Parentage test

Introduction

Dog breeds are improved for various purposes, and more than 400 breeds are distributed in the world. These breeds have peculiar shapes and characteristics, which have been based on pure breed management. Since parentage testing can be a basic method for scientific and pure breed control, many studies for dog parentage testing have been performed [1-3, 5-8, 10].

Recently microsatellite analysis is introduced for dog parentage testing. DNA microsatellites are short and randomly repeated sequence units with a distribution throughout the genome. The repeated units are bi-, tri-, or tetranucleotide [4, 9]. Microsatellites are highly polymorphic, and they are stable enough to be inherited unchanged from one generation to the next [2, 4, 12]. To detect microsatellite polymorphisms for specific gene targets, genomic DNA are amplified by PCR and performed by Gene analysis [2, 4, 9].

When two alleles show as co-dominant, one is of maternal origin and the other is of paternal origin. Therefore, offspring must have some alleles originating from the mother or the father for identifying the parentage relationship between parents and offspring. Due to this reason, microsatellite analysis is useful for parentage testing [3], breed differentiation [1, 2, 4] and sex [11].

This study was conducted to determine whether the application of 22 ISAG (International Society for Animal Genetics) canine markers is available for parentage testing in Beagle dogs.

Materials and Methods

Eight Beagles, which are 1 mother dog, 4 candidate father dogs, and 3 offsprings were bred at Cheju National University. Twenty-two ISAG canine microsatellite markers were used for parentage testing (Table 1).

DNA was extracted from the blood by the phenol/chloroform extraction method and was amplified on

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Table 1. Characterization of 22 ISAG canine microsatillite markers

CFA	Size range	Locus	Dye	Forward Sequence	Reverse Sequence
CFA23	277-297	AHTk253	FAM	ACATTTgTgggCATTggggCTg	TgCACATggAggACAAgCACgC
CFA13	68-118	AHT121	FAM	TATTgCgAATgTCACTgCTT	ATAgATACACTCTCTCTCCg
CFA12	135-179	FH2054	NED	gCCTTATTCATTgCAgTTAggg	ATgCTgAgTTTTgAACTTTCCC
CFA22	109-133	C22.279	NED	TgCTCAATgAAATAAgCCAgg	ggCgACCTTCATTCTCTgAC
CFA21	87-111	INRA21	PET	ATgTAgTTgAgATTTCTCCTACg	TAATggCTgATTTATTTggTgg
CFA26	83-101	AHTk211	VIC	TTAgCAgCCgAgAAATACgC	ATTCgCCCgACTTTggCA
CFA18	224-242	REN54P11	FAM	gggggAATTAACAAAgCCTgAg	TgCAAATTCTgAgCCCCACTg
CFA07	192-212	REN162C04	PET	TTCCCTTTgCTTTAgTAggTTTTg	TggCTgTATTCTTTggCACA
CFA16	236-254	AHTh260Ren	PET	CgCTATACCCACACCAggAC	CCACAgAggAAgggATgC
CFA06	215-239	AHTh171	VIC	AggTgCAgAgCACTCACTCA	CCCATCCACAgTTCAgCTTT
CFA11	231-249	REN105L03	FAM	ggAATCAAAAgCTggCTCTCT	gAgATTgCTgCCCTTTTTACC
CFA36	111-141	AHTH130	NED	gTTTCTCTCCCTTCgggTTC	gACgTgTgTTCACgCCAg
CFA29	154-170	REN169O18	NED	CACCCAACCTgTCTgTTCCT	ACTgTgTgAgCCAATCCCTT
CFAX	182-217	Amelogenin	NED	gTgCCAgCTCAgCAgCCCgTggT	TCggAggCAgAggTggCTgTggC
CFA34	139-155	REN64E19	PET	TgTATTTTAATgTggCAgTTT	gACAAggACAggCAATACAgT
CFA14	199-221	REN169D01	PET	AgTgggTTTgCAAgTggAAC	AATAgCACATCTTCCCCACg
CFA02	228-244	FH2848	VIC	CAAAACCAACCCATTCACTC	gTCACAAggACTTTTCTCCTg
CFA11	126-156	AHT137	VIC	TACAgAgCTCTTAACTgggTCC	CCTTgCAAAgTgTCATTgCT
CFA15	268-282	REN247M23	VIC	TggTAACACCAAggCTTTCC	TgTCTTTTCCATggTggTgA
CFA33	104-136	INU005	FAM	CTTTCTACCAgCAAggTTAC	TTCCCATTTAATTgCCTCT
CFA12	143-157	INU030	FAM	ggCTCCATgCTCAAgTCTgT	CATTgAAAgggAATgCTggT
CFA10	204-220	INU055	FAM	CCAggCgTCCCTATCCATCT	gCACCACTTTgggCTCCTTC

Table 2. The results of parentage test in candidate male Beagles

Sample*	AHTk253	AHT121	FH2054	C22.279	INRA21	AHTk211	REN54P11	REN162C04	AHTh260Ren	AHTh171	REN105L03
F9	285/289†	93/99	154/166	123/129	92/98	87/91	227/237	201/205	240/248	231/231	240/242
P1	289/289	93/103	154/166	117/123	92/98	87/97	227/237	201/207	240/248	231/231	240/240
P2	285/289	91/93	154/162	115/123	98/98	87/97	237/237	201/207	240/240	223/231	236/242
P3	289/289	91/99	166/166	117/123	92/92	87/89	227/237	201/205	240/248	231/231	236/240
M1	285/289	99/99	154/154	123/123	92/92	87/87	227/229	201/201	242/248	223/223	232/242
M5	289/289	103/103	154/158	115/123	92/96	87/95	227/239	205/207	244/250	223/231	232/242
M6	289/289	103/103	154/158	115/123	92/96	87/95	227/239	205/207	242/244	217/231	234/242
M7	285/289	91/103	162/166	115/117	92/98	89/97	227/237	201/207	240/248	223/231	236/240

*F: mother dog; P: offspring; M: candidate male; †PCR product size for allele.

Table 2. continued

Sample*	AHTh130	REN169O18	Amelogenin	REN64E19	REN169D01	FH2848	AHT137	REN247M23	INU005	INU030	INU055
F9	126/128†	159/167	217/217	138/144	200/214	234/242	141/145	273/273	109/123	148/148	211/215
P1	126/128	159/159	217/217	138/144	214/218	234/242	133/141	271/273	123/125	148/148	209/215
P2	126/128	159/159	217/217	138/144	200/218	234/240	133/145	271/273	109/123	148/148	211/211
P3	126/128	167/167	217/217	144/144	200/200	234/242	133/141	271/273	123/125	142/148	209/215
M1	126/126	161/165	179/217	138/144	200/200	234/240	135/135	273/273	109/129	148/148	211/213
M5	126/126	161/161	179/217	138/144	200/200	238/238	131/135	269/271	123/129	142/148	209/213
M6	116/126	161/161	179/217	138/144	200/200	238/238	131/143	269/271	109/123	142/142	209/213
M7	126/128	159/167	179/217	138/144	200/218	234/240	133/141	271/271	123/125	142/148	209/211

*F: mother dog; P: offspring; M: candidate male; †PCR product size for allele.

Perkinelmer 9400 (Perkinelmer, USA). PCR was performed in 10 ul of reaction buffer containing 10 pmols of each primer, 30 ng DNA, 0.07 of Solget Tag (0.175 u). Optimizing conditions are controlled for each microsatellite by adjusting Band Doctor volume. Standard PCR conditions were performed by Touch Down program (65°C-55°C). PCR products for alleles were run on an ABI PRISM 3130x (Applied Biosystems, USA) Genetic Analyzer and analyzed by GeneScan Analysis (*Ver* 3.0) and Genotyper (*Ver* 2.1) software.

Results

We confirmed parentage relationship between 3 offsprings and 4 candidate fathers through analysis of allele typing results for 22 ISAG microsatellite markers in 8 Beagle dogs (Table 2).

Candidate fathers M1, M5, and M6 could be excluded as the father because of mismatching alleles which 3 offspring had in FH2054, INRA21, AHTk211, REN54P11, AHTh260Ren, AHTh171, REN105LO3, AHTh130, REN169O18, FH2848, AHT137, REN 247M23, INU055.

Three offspring have alleles, which didn't originate from the mother in AHT121, FH2054, C22.279, AHTk211, REN162C04, AHTh171, REN105L03, REN 169D01, FH2848, AHT137, REN247M23, INU005, INU030, INU055. We could confirm that the alleles originated from candidate M7. In addition, M7 had alleles that matched with 3 offspring in the rest of the markers. From these results, we concluded that candidate M7 is a real father. The results of locus (amelogenin) which were designed for sex distinction matched with the real gender of the 8 Beagle dogs (female; 217/217 homozygosity, male; 179/217 heterozygosity).

Discussion

The present study demonstrates a new approach that enables effective dog parentage testing using 22 ISAG microsatellite markers. Recently this approach based on genetic polymorphism was also used for parentage testing of human beings [6]. Specifically, it is possible to identify parentage relationship with more than 99.99% accuracy through allele analysis. In addition, correct parentage testing is needed for systemic pure breed management [3, 5, 7, 8, 10, 12].

Tested 22 ISAG microsatellite markers are very accurate for Beagle dog parentage testing, because each allele, which offspring contain, must match with some alleles originating from the mother or the father or both of them in all 22 microsatellite markers. From our data, we confirmed that 22 ISAG microsatellite markers for target loci used in this study are useful tools for parentage testing.

In addition, Amelogenin is an applicable marker for detecting real sex in dogs [11]. Twenty-two ISAG microsatellite markers will be valuable markers for managing to breed pure-bred dogs.

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