Genomic Polymorphism Analysis using Microsatellite Markers in Gyeongju Donggyeong Dogs

Seung-Chang Kim[a], Lee-Kyung Kim[a], Seog-Kyu Choi[a,1], Chang-Min Park, Sun-Ae Park, Yong-Min Cho, Dajeong Lim, Han-Ha Chai, Seung-Hwan Lee, Ji-Woong Lee[2], Sang-Soo Sun[2] and Bong-Hwan Choi[†]

Division of Animal Genomics & Bioinformatics, National Institute of Animal Science, RDA, Suwon 441-706, Republic of Korea

[1]Institute of Conservation Gyeongju Donggyeong Dog, Dongguk, Gyeongju 780-714, Korea

[2]Devision of Animal Science, Insti. of Ag. Sci. and Tech., Chonnam National University, Chonnam 500-757, Korea.

ABSTRACT

This study was conducted to find a useful marker for gene polymorphism analysis using Microsatellite marker (MS marker) in Gyeongju Donggyeong dog. Twenty three MS marker analyzed the genetic features of DNA using 100 Gyeongju Donggyeong dogs in Gyeongju area. It was performed multiplex PCR with 3 set primer divided 9, 10 and 4 by analysis of conditions among MS markers. The results were calculated heterozygosity, polymorphic information content (PIC), allele frequency and number of allele at each locus using Microsatellite Toolkit software and Cervus 3.0 program. Total 148 alleles were genotyped to determine and average 6.43 alleles was detected. FH3381 had the highest of 15 alleles and FH2834 had the lowest of 2 alleles. Expected heterozygosity had a wide range from 0.282 to 0.876 and had average value of 0.6496. Also, Observed heterozygosity had a more wide range from 0.200 to 0.950 and had average value of 0.6404. PIC had range from 0.262 to 0.859 and average PIC was calculated 0.606. Especially, FH2998 represented the highest rate of observed heterozygosity of 0.950 and FH3381 represented the highest rate of expected heterozygosity of 0.876 and PIC of 0.859. The use of these markers was considered to be useful to study genetic traits of Gyeongju Donggyeong dog.

(Key words : Microsatellite marker, Canine, Gyeongju Donggyeong dog, Allele, Heterozygosity)

INTRODUCTION

The dog known the first domesticated people is being raised for a variety of purposes, such as pet dog, hunting dog, cattle dog, rescue dog and army dog. The various breeds have been selected in accordance with a person's purpose, environment or preference because diversity of size, shape, physique and the nature according to breed. It exist various dog association to keep a record of the lineage and to preserve important lineages by increasing number of people preferring thoroughbred dogs. Typically, canine association was the American Kennel Club (AKC), the British Kennel Club (KC) and the Federation cynologique internationale (FCI). It aim to protect thoroughbred dogs and to the establishment of the breed standard, the breed registration and the issuance of pedigree. The registered pedigrees by these associations received trust with guarantee of thoroughbred dogs and lineage. Recent years, attention on the pet was increased according to progress of urbanization, nuclear families and aging in the society. So, it was increasing to nurture dog. Associations organized in Korea, large and small, but pedigree issued by its not received the authority and trust, such as a foreign country. The reason was that breeder was suspected registering the wrong lineage relationships for the purpose of personal profit because reported pedigree by breeder been only recognized. This distrust spreading in most of the native dog as well as Jindo dog worked a big obstacle a healthy supply, maintain and fixing business of lineage of native dog (Chae *et al*., 1998). New standards which can be delimited the definitive lineage are needed to solve these problems.

The Jindo dog and Sapsal dog designated as a natural monument, and Jeju dog, Poongsan dog, Bool dog and Gyeongju Donggyeong dog is Korea native breed (Ha and Kim, 1998). From among these, Gyeongju Donggyeong dog, indigenous dog of Gyeongju local, have physically characteristics with indicating the form of short

---

[a] These authors contributed equally to this work.

* This work was supported by the "High-throughput sequencing and variation for genetic markers development in Korean native animals" project of the National Institute of Animal Science, RDA, Korea.

[†] Corresponding author : Phone: +82-31-290-1592, E-mail: bhchoi@korea.kr

or non-tail (Lee *et al*., 2008). The name of Gyeongju Donggyeong dog is originated from Dongkyung, another name of Gyeongju, the capital of ancient Shila kingdom in Korean (Cho *et al*., 2008). Being in Dongkyung local of the 12th and the 17th century remains record of Samguk Sagi (History of the Three Kingdoms) and the fact which was bred around the Gyeongju area until the early 20th century was identified. This is shown that Gyeongju Donggyeong dog had been well adapted to climate of Korea and was been native breed while maintaining the characteristics. At the anatomical evaluation, congenital taillessness (anury) represented 4 or less tailbones and tail-shortness (brachyury) represented 5~10 tailbones. But shepherd had 20~22 tailbones and Jindo had 20 tailbones typically. Gyeongju Donggyeong dog was confirmed normal form historically, physically and functionally but anury and brachyury may be caused by mutations (Wansbrough, 1996; Haworth *et al*., 2001; Cho and Kim, 2006). Gyeongju Donggyeong dog (Gyeongju dog) especially known with the oldest history of native dog but it was not properly controlled pedigree management and specification management due to the negligence of the management. In recent years, the trend elucidates relationship with other species through a phylogenic analysis along with identification of genetic characteristics with the molecular genetic studies for pedigree management and preservation, and is the research about the origin and evolution (Tanabe *et al*., 1991; Ha *et al*., 1998; Cho and Cho, 2006). In addition, DNA analysis technique using MS marker is being applied in paternity diagnosis, individual identification, the preservation of endangered species, the phylogeny associated with origin and the history tracking system in various countries of the world (Marklund *et al*., 1994; Usha *et al*., 1995; Mommens *et al*., 2002; Villanueva *et al*., 2002; Cho *et al*., 2003; Yoon *et al*., 2005). Together our previous study (Kim *et al*., 2011), this study was conducted to find a useful marker for verify of genetic similarity of breed populations, phylogenic study, the relationships between groups within breeds through gene polymorphism analysis using MS marker in Gyeongju Donggyeong dog.

MATERIALS AND METHODS

Animals and Extraction of Genomic DNA

Genomic DNA was extracted from blood of total 100 animals by Gyeongju Donggyeong dog using Wizard genomic DNA purification kit (Promega, USA) according to the manufacturer's protocol and analyzed concentration and purity at absorbance of 260 nm and 280 nm using ND-1000 spectrophotometer (Nanodrop, USA).

MS Marker for Allele Analysis

MS markers utilized this study were firstly selected 228 MS markers based on MS genetic loci of dog reported Mapviewer database of NCBI (National Center for Biotechnology Information) and were selected 45 MS markers considered annealing temperature of 61℃, product size and type of dye for Gradient PCR thereafter. The selected MS markers divided into 3 set of each 15 and were composed of the final set of 9, 10 and 4 that satisfied condition of multiplex PCR. Total 23 primers of 3 set are shown in Table 1.

Multiplex PCR and MS Analysis

Multiplex PCR was set up in 25 ul reaction volume consisting 6 ul (20 ng/ul) of genomic DNA, 0.4 ul (10 pmole) each of fluorescence dye primer of forward and reverse, 1 ul (Unit/ul) of Hot Start Taq DNA polymerase, 4 ul of 10× buffer and 3 ul of 2.5 mM dNTP. Conditions of Thermal Cycler PTC-0240 (MJ Research, Inc., MA, USA) were as follows: 15 min at 95℃ for initial denaturation, followed by 5cycles with denaturation at 95℃ for 60 sec, annealing at 62℃ for 75 sec and elongation at 72℃ for 60 sec, 5cycles with denaturation at 95℃ for 60 sec, annealing at 61℃ for 75 sec and elongation at 72℃ for 60 sec, 25cycles with denaturation at 95℃ for 60 sec, annealing at 60℃ for 75 sec and elongation at 72℃ for 60 sec. The final had a extension temperature of 65℃ for 30 min. PCR products were analyzed using the ABI-3730XL genetic analyzer (Applied Biosystems, USA) and GeneMapper version 4.0 (Applied Biosystems, USA).

Statistic analysis

Alleles of MS marker from Genotyper Software was organized by analyzing using Microsatellite Toolkit software (Park, 2000) and Cervus 3.0 program (version 3.0, The University of Edinburgh) (Marshall *et al*., 2002). The Heterozygosity, PIC (Polymorphic information content), allele frequency and number of allele at each locus were calculated from Gyeongju Donggyeong dog. Heterozygosity showed up variety of allele in marker through calculated the value of expected and observed heterozygosity.

RESULTS AND DISCUSSION

This study investigated genetic characteristics based on the frequency of the genetic diversity of microsatellite DNA from 100 animals judged Gyeongju Donggyeong dog. MS marker was used 23 marker selected by analysis of conditions among MS marker of dog reported in the Mapviewer database. Amplified product showed a variety sizes to 139 bp from 390bp and num-

Table 1. List of microsatellite markers and primer

Set No.	Chr.	Marker	Forward(5'→3')	Reverse(3'→5')	Annealing temp.	Product size	Dye
Set 01	10	FH2537	AAAAAGTGTAGAGCTTTCTTCAAA	ATTGAGACCCAAGACTGTTAGTG	61℃	146~176	Fam
	2	FH3005	ACTCATTTCCAAGGTGATTTG	GTACTCACCGCAAGTGCAAG		200~236	Fam
	12	FH3116	GAGAAATCCTGTCATGTGCTG	CCTTTTCCCTTCTTTCCTTG		186~200	VIC
	19	FH3372	AGTGCCTTTGAATGTTAATGC	ACATCAAAATGGTTACACTTGG		142~162	VIC
	10	FH3381	CCCAGAAACTCAACTGATGC	AGCTCTTACACGCATTGAGG		276~312	Fam
	10	FH3921	CCTTCTTCTTAACACCTCTTCC	CTCTGTTTGCCAGATGATAACC		364~394	NED
	30	REN51C16	CAGTTCATCCTTCCCCCTCTC	GTGCTAGTCTGGCTGTGCTCA		246~264	VIC
	26	REN62M06	AAGTGGAATGGAGTCTGC	CATGAACCTGTCGTAAGC		243~255	NED
	27	REN277O05	CCTCCTCTCACTTGTCCTGC	AAATGGTGTCTTCAGCTCCG		331~338	VIC
Set 02	9	FH1014	AGGCTATTAACCCCTGATCG	CGATGCCTTACTTAAACAAACC	61℃	242~250	Fam
	4	FH2097	CAATGTCGAATTCCATGGTG	ATGGAGCAAGATGTGTTTGTG		268~300	NED
	X	FH2584	GTTAGGTTCACAGTGGGCGT	ACTCAAAGACCTGGAGGGGT		299~317	VIC
	31	FH2712	AAGGTAGTCCCACGATCCTC	GAGCCCTGTTCTCAGGTTG		170~186	PET
	18	FH2834	GCAAGCTTTAAAATACCTTTCC	GCCTGAACTGATTGATGACC		263~265	Fam
	36	FH2998	GATTTTGATACCCTGAGAATGC	CTCACTGGCTCTCACATGC		196~228	PET
	16	FH3058	GCCTTCCATAGATGAATGAGG	CCATACATGGTTTTGAGAACG		218~234	Fam
	35	REN112C08	ATGGCCCACCGATACACA	TCGGGGACATACTTGAACC		218~236	NED
	Y	REN197E16	TGGGTGTGAGTCATCCAAGA	CGTTACTGTATGCTTAAGCTTTTGA		140~160	Fam
	8	REN204K13	TCGGGATGTTTCTCTTCCAC	CTGCTTAAATTCTCCCAGCG		246~254	VIC
Set 03	12	FH2054	GCCTTATTCATTGCAGTTAGGG	ATGCTGAGTTTTGAACTTTCCC	61℃	148~180	Fam
	24	FH2079	CAGCCGAGCACATGGTTT	ATTGATTCTGATATGCCCAGC		269~293	NED
	31	FH2582	TGGAGTGTGTTCCAAGGTCA	GTTGTTCCCACAAAAGGCAG		342~386	NED
	26	REN01O23	TTCCCTGCAGCCCTTCCTCA	TGTGCCTCATTCCTTTTTAT		185~203	VIC

ber of alleles was detected of 2~15 (average 6.43). It was performed multiplex PCR with 3 set primer divided 9, 10 and 4. Then, multiplex PCR results using 23 MS marker were calculated heterozygosity, PIC, allele frequency and a number of allele at each locus using Microsatellite Toolkit software and Cervus 3.0 program. The number of allele and the gene frequency of allele on MS marker are shown in Table 2. Total 148 alleles were genotyped to determine and average 6.43 alleles was detected. The first set consists of 9 MS marker comes out 67 alleles. Especially, FH3381 has the highest of 15 alleles and REN62M06 has the lowest of 3 alleles. The second set consists of 10 MS marker comes out 55 alleles. FH2998 have the highest of 10 alleles and lowest (FH2834) emerged as having 2 alleles. The final set consists of 4 MS marker shows 26 alleles. FH2582 have the highest of 11 alleles and lowest (FH2079 and REN-01O23) emerged as having 4 alleles. As a result, total 148 alleles were genotyped to determine.

The observed and expected heterozygosity, and PIC were calculated to determine the genetic diversity, the results are shown in Table 3. The average observed heterozygosity of marker locus was 0.6404 and the average expected heterozygosity was 0.6496. Especially, FH-2998 represented the highest rate of observed heterozygosity of 0.95 and FH2079 represented the lowest rate of observed heterozygosity of 0.2. Also, FH3381 represented the highest rate of expected heterozygosity of 0.876 and REN62M06 represented the lowest rate of expected heterozygosity of 0.282. Other studies were reported that discrimination of the breed over 96% showed by marker of a similar level in pig and cattle (Fan *et al*., 2005; Oh *et al*., 2008). Each of these marker sets, heterozygosity by various alleles also derived the value

Table 2. Allele frequency of the 23 microsatellite markers in 100 Gyeongju Donggyeong dogs

Marker	No. of alleles	Allele (Gene frequency)						
FH2537	10	145 (0.28)	150 (0.16)	154 (0.04)	158 (0.045)	160 (0.14)	164 (0.175)	168 (0.105)
		172 (0.01)	176 (0.02)	180 (0.025)				
FH3005	6	204 (0.065)	208 (0.565)	212 (0.09)	216 (0.04)	220 (0.205)	224 (0.005)	
FH3116	4	186 (0.02)	190 (0.82)	192 (0.115)	196 (0.045)			
FH3372	6	142 (0.03)	150 (0.19)	154 (0.44)	158 (0.18)	162 (0.135)	166 (0.025)	
FH3381	15	273 (0.005)	277 (0.2)	281 (0.15)	285 (0.185)	289 (0.045)	293 (0.07)	295 (0.025)
		299 (0.025)	301 (0.13)	303 (0.01)	305 (0.055)	307 (0.02)	311 (0.005)	315 (0.05)
		319 (0.025)						
FH3921	12	362 (0.005)	366 (0.005)	368 (0.025)	370 (0.075)	372 (0.015)	374 (0.205)	376 (0.035)
		378 (0.16)	380 (0.03)	382 (0.335)	386 (0.065)	390 (0.045)		
REN51C16	6	246 (0.025)	248 (0.505)	250 (0.135)	256 (0.16)	260 (0.005)	262 (0.17)	
REN62M06	3	243 (0.84)	245 (0.06)	253 (0.1)				
REN277O05	5	312 (0.09)	335 (0.595)	337 (0.19)	339(0.03)	341 (0.095)		
FH1014	4	244 (0.025)	246 (0.76)	248 (0.14)	250 (0.075)			
FH2097	8	272 (0.08)	276 (0.02)	280 (0.105)	284 (0.11)	288 (0.175)	292 (0.055)	296 (0.395)
		298 (0.06)						
FH2584	6	294 (0.21)	300 (0.055)	302 (0.31)	306 (0.065)	308 (0.275)	314 (0.085)	
FH2712	8	173 (0.175)	175 (0.17)	177 (0.235)	179 (0.035)	181 (0.085)	183 (0.09)	185 (0.19)
		187 (0.02)						
FH2834	2	263 (0.665)	265 (0.335)					
FH2998	10	196 (0.225)	204 (0.05)	212 (0.13)	216 (0.185)	220 (0.08)	224 (0.115)	228 (0.16)
		232 (0.01)	236 (0.025)	240 (0.02)				
FH3058	4	222 (0.495)	224 (0.045)	228 (0.415)	230 (0.045)			
REN112C08	3	218 (0.385)	220 (0.3)	234 (0.315)				
REN197E16	5	139 (0.005)	141 (0.715)	143 (0.055)	145 (0.16)	149 (0.065)		
REN204K13	5	246 (0.405)	248 (0.48)	250 (0.03)	254 (0.08)	256 (0.005)		
FH2054	7	150 (0.21)	154 (0.05)	158 (0.195)	162 (0.13)	166 (0.09)	170 (0.2)	174 (0.125)
FH2079	4	269 (0.64)	273 (0.22)	277 (0.13)	281 (0.01)			
FH2582	11	342 (0.06)	350 (0.195)	354 (0.065)	358 (0.03)	362 (0.05)	366 (0.055)	370 (0.26)
		374 (0.015)	378 (0.07)	382 (0.085)	386 (0.105)			
REN01O23	4	185 (0.525)	191 (0.305)	199 (0.165)	207 (0.005)			

of a relatively wide range, but are distributed for each set. The average PIC was calculated 0.606. Normally, it was known to be high reliability of the marker for the pedigree analysis if PIC value was greater than 0.5000 and microsatellites of PIC > 0.7000 were best for linkage analysis (Hearne *et al*., 1992). Markers of PIC > 0.5 out of the 23 markers were 16 and markers of PIC > 0.7 were 9. Especially, FH2054, FH2537, FH2582, FH2712, FH2998 and FH3381 had a value over the 0.8. Six kinds marker of more than 0.8 had been shown to be useful markers to Gyeongju Donggyeong dog study. Many alleles and the PIC exhibited by MS marker shown high polymorphisms.

The most urgent problem for conservation and breeding of native dog is to find out genetic structure identification and differentiation existing within each group. It will be made a more scientific and efficient breeding if standard of native dog be made to establish and to distinct at the level of the gene. Therefore, allele analysis using these MS markers had recognized as effective and useful tool for investigation of paternity diagnosis and individual identification through genetic

Table 3. Expected heterozygosities and Observed heterozygosities and PIC value at 23 microsatellite in Gyeongju Donggyeong dogs

Set	Locus	Ob H	Ex H	PIC
Set 1	FH2537	0.820	0.834	0.809
	FH3005	0.381	0.604	0.599
	FH3116	0.350	0.314	0.291
	FH3372	0.730	0.722	0.679
	FH3381	0.890	0.876	0.859
	FH3921	0.890	0.809	0.782
	REN51C16	0.720	0.675	0.631
	REN62M06	0.280	0.282	0.262
	REN277O05	0.580	0.595	0.552
Set 2	FH1014	0.460	0.399	0.366
	FH2097	0.820	0.781	0.753
	FH2584	0.470	0.774	0.734
	FH2712	0.940	0.836	0.810
	FH2834	0.470	0.448	0.346
	FH2998	0.950	0.854	0.832
	FH3058	0.550	0.582	0.491
	REN112C08	0.700	0.666	0.589
	REN197E16	0.350	0.458	0.422
	REN204K13	0.640	0.601	0.517
Set 3	FH2054	0.900	0.839	0.813
	FH2079	0.200	0.528	0.470
	FH2582	0.919	0.858	0.839
	REN01O23	0.720	0.607	0.533

Ex H: Expected heterozygosity, Ob H: Observed heterozygosity, PIC: Polymorphic information content

traits from Gyeongju Donggyeong dog.

REFERENCES

1. Chae YJ, Lee BC, Lee H (1998): Paternity test in dogs by DNA analysis. Korean J Vet Clin Med 15:274-278.
2. Cho BW, Cho GJ (2006): Physical characteristics and microsatellite polymorphisms in miryang native dogs. J life Sci 16:626-631.
3. Cho GJ, Cho BW, Kim SK, Lee KW, Kim YK (2003): Analysis of microsatellite DNA polymorphism for parentage testing in dog breeds. J Anim Sci & Technol 45:191-198.
4. Cho S, Park Y, Lee E, Lee H, Kim O (2008): Study on Dongkyung-i dog characterized as anury and brachyury. Journal of Veterinary Clinics-Seoul-25, 15.
5. Cho SJ, Kim OJ (2006): A congenital anury in Korean native dogs. Laboratory Animal Research 22:187-188.
6. Fan B, Chen Y, Moran C, Zhao S, Liu B, Yu M, Zhu M, Xiong T, Li K (2005): Individual-breed assignment analysis in swine populations by using microsatellite markers. Asian-Aust J Anim Sci 18:1529.
7. Ha JH, Kim KS (1998): A review on the origin of Korean native dags. Korean J Anim Sci 40:701-710.
8. Ha JH, Lee SE, Tak YB, Kim JB (1998): The physical characteristics and blood proteins of Korean native dogs. Korean J Anim Sci 40:711-720.
9. Haworth K, Putt W, Cattanach B, Breen M, Binns M, Lingaas F, Edwards YH (2001): Canine homolog of the T-box transcription factor T; failure of the protein to bind to its DNA target leads to a short-tail phenotype. Mammalian genome 12:212-218.
10. Hearne CM, Ghosh S, Todd JA (1992): Microsatellites for linkage analysis of genetic traits. Trends in Genetics 8:288-294.
11. Kim SC, Jang HC, Kim LK, Lim DJ, Lee SH, Cho YM, Kim TH, Seong HH, Oh SJ, Choi BH (2011): Paternity diagnosis using the multiplex PCR with microsatellite markers in dogs. Reprod Dev Biol 35: 399-405.
12. Lee EW, Choi SG, Cho GJ (2008): Analysis of microsatellite DNA polymorphisms for pedigree verification in Kyungju Dog(Dongkyung-i). J life Sci 18: 902-906.
13. Marklund S, Ellegren H, Eriksson S, Sandberg K, Andersson L (1994): Parentage testing and linkage analysis in the horse using a set of highly polymorphic microsatellites. Animal Genetics 25:19-23.
14. Marshall T, Slate J, Kruuk L, Pemberton J (2002): Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology 7: 639-655.
15. Mommens G, Van Zeveren A, Peelman L (2002): Effectiveness of bovine microsatellites in resolving paternity cases in American bison, *Bison bison* L. Animal Genetics 29:12-18.
16. Oh JD, Lee JA, Kong HS, Park KD, Yoon DH, Jean GJ, Lee HK (2008): Estimation of genetic characteristic and cumulative power of breed discrimination using microsatellite marker in Hanwoo. J Emb Trans 3:203-209.
17. Park DDE (2000): Microsatellite toolkit for MS excel 97 or 2000.
18. Tanabe Y, Ôta K, Ito S, Hashimoto Y, Sung YY, Ryu JK, Faruque MO (1991): Biochemical-genetic relationships among Asian and European dogs and the ancestry of the Japanese native dog. Journal of

Animal Breeding and Genetics 108:455-478.
19. Usha A, Simpson S, Williams J (1995): Probability of random sire exclusion using microsatellite markers for parentage verification. Animal Genetics 26: 155-161.
20. Villanueva B, Verspoor E, Visscher P (2002): Parental assignment in fish using microsatellite genetic markers with finite numbers of parents and offspring. Animal Genetics 33:33-41.
21. Wansbrough RK (1996.): Cosmetic tail docking of dogs. Aust vet J 74:59-63.
22. Yoon DH, Kong HS, Oh JD, Lee JH, Cho BW, Kim JD, Jeon KJ, Jo C, Jeon GJ, Lee HK (2005): Establishment of an individual identification system based on microsatellite polymorphisms in Korean Cattle (Hanwoo). Asian-Aust J Anim Sci 18: 762-766.

(Received: 1 November2012/ Accepted: 20 December 2012)