Genetic Diversity Analysis of the Cheju Horse Using Random Amplified Polymorphic DNAs

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This experiment was carried out to analyze genetic characteristics and to develop the breed specific DNA marker for Cheju-native horse. If this marker contains high repetitive sequences, it is possible to convert a RAPD marker of interest into a single-locus PCR marker called a sequence characterized amplified region(SCAR). Twenty six Cheju-native horse and Fifty thoroughbred genomic DNA were pooled and PCR. were accomplished using 800 random primers. Comparing the pooled DNA from Cheju-native horse and thoroughbred, we found 9 primers which identified markers present in the pooled DNA from breed but absent in the other breed. Among 9 random primers, 6 primers were thoroughbred specific and 3 primers were Cheju-native horse specific. Testing individual horse revealed that 5 marker showed the similar band pattern between Cheju-native horse and Thoroughbred. However, 4 marker were wholly absent in breed while present in the other breed. UBC 126_{3500bp}, UBC 162_{500bp}, and UBC 244_{1200bp} was detected only Thoroughbred and UBC 562_{560bp} was detected Cheju-native horse, respectively. After determining of the cloned breed-specific fragment sequence, we designed the SCAR-primers and carried out PCR. Compared to random primer, RAPD-SCAR primer didn't show significantly higher specific band. However, RAPD analysis is useful for genetic characterization of Cheju-native horse.

Key words – Cheju-native horse, Thoroughbred, RAPD-PCR, SCAR

The evolutionary origin and classification of Cheju-native horse have been attracted to us for a long time. Knowledge of the genetic relationships between Cheju-native and other breed horses is valuable for use in breeding program and conservation of genetic resource.

Cheju-native horse has been well adapted to the rough environment of Cheju island for a long centuries. Living primarily in the mid-mountain area, Cheju-native horse is often compared with the Cheju people whose common feature is small but strong physique being able to overcome the extreme weather. These notable characteristics of Cheju-native horse has made them to be an enduring symbol of Cheju-do, Korea.

The advent of improved transportation and farming methods led to a general decline in the Cheju-native horse population. To preserve this important historical treasure, Government designated them Natural Monument No.347. As a further commitment to their preservation, Cheju racecourse was constructed to preserve and foster Cheju-native horse through pony racing. For detecting polymorphic markers in plants and animals, randomly amplified polymorphic DNA(RAPD) assay based on the polymerase chain reaction(PCR) utilize a short oligonucleotide primers of arbitrary sequences to amplify discrete regions of the genome[9-11]. On the basis of numerous successful applications in domestic animals such as cattle, chicken, dog, horse, mice and sheep, the RAPD assay has potential to play a useful role in genetic analysis of livestock species[2-4,6]. Especially in the horse, RAPD marker feasible to distinguish the breed between Thoroughbred and Arabian horses[1]. In the past, the characteristics of Cheju-native horse had been based on the observation of physical appearances such as body conformation, coat color, body lengths, etc. Characteristics of Cheju-native horse were studied on the blood protein polymorphism for lately several years and recently analyzed the D-loop region of mtDNA between breed[5,7].

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Materials and Methods

Animals and Genomic DNA preperation

26 Cheju-native horse and 50 Thoroughbred horse blood samples were collected. Genomic DNA extraction was processed according to the procedure[9].

PCR primer and condition

Random oligonucleotide was prepared from UBC(University of British Columbia, Canada). PCR including 10

pmole random primer, 1× reaction buffer(50 mM KCl, 10 mM Tris-Cl, 1.5 mM $MgCl_2$), 200 μM dNTP mixture, 50 ng/μl of template DNA and 1 unit Taq DNA polymerase(Takara, Japan) was accomplished by a modified procedure described[11]. After denaturation at 94℃ for 5 min, 50 cycles at 94℃ for 1 min, 40℃ for 1 min, 72℃ for 2 min were fulfilled and then post-elongation at 72℃ for 10 min. in a GeneAmp PCR system 9600(perkin Elmer, USA).

Breed-Specific Band Cloning and Sequencing

Cloning of the amplified breed specific fragment was processed according to the procedures of the pGEM-T Easy Vector System(Promega, USA). Plasmid DNAs including breed-specific bands were sequenced by Perkin Elmer dye terminator sequencing Kit(PE Applied Biosystems) using Perkin Elmer automatic sequencer(ABI377; PE Applied Biosystems).

Design of SCAR primer

After the cloned breed-specific DNA fragment sequencing, strand-specific oligonucleotide primers which added to the 3'-terminus of random primer(8 to 14 bp) were designed based on the sequence information(Bioneer Co. Korea).

Results and Discussion

Comparing the pooled DNA from Cheju-native and Thorougbred horse, we found breed specific primers(UBC 15, UBC 18, UBC 162, UBC 213, UBC 234, UBC 443, UBC 562, and UBC 754) which identified markers present in pooled DNA from breed but absent in the other breed (Fig. 1). Among nine random primer, five primers (UBC 151600bp, UBC 18700bp, UBC 162550bp, UBC 2441700bp, and UBC 4431100bp) were Thoroughbed specific and five primer (UBC 18400bp, UBC 213850bp, UBC 2341300bp, UBC 562500bp and UBC 754350bp) were Cheju-native horse specific. Among these primer, UBC 18 simultaneously showed the Thoroughbred specific fragment, 700 bp, Cheju-native specific fragment, 400 bp, respectively. Testing individual horse revealed that random primers showed the similar RAPD fingerprint between Cheju-native horse and Thoroughbred. For example, UBC 4431000bp detected 66.0% in the Thoroughbed and 38.5% in the Cheju-native horse, respectively. However, four primers were wholly absent in preed while present in the other breed while present in the other breed, UBC 1263500bp, UBC 162500bp, and UBC 2441200bp, detected only in Thoroughbred and but UBC 5621500bp showed in Cheju-native horse(Fig. 2). UBC 851500bp and UBC 851700bp absent in Thoroughbred but partly present in Arabian horse(11/31) and UBC1261000bp was not detected in the Thoroughbred but detected in the Arabian horse and it is the critical fragment between two breed.[1] Compared to above report, UBC 851700bp and UBC 1261000bp fragment was detected in the Thoroughbred (56/56) and Cheju-native horse(26/26) (Fig. 3). The reason for inaccordance with this result suggested that origin of Thorouthbred in korea somewhat differ from Bailey and Lear group And similar pattern between Throuthbred and Cheju-native horse hourse imply that genetic distance between two breed is quite close. To make the SCAR

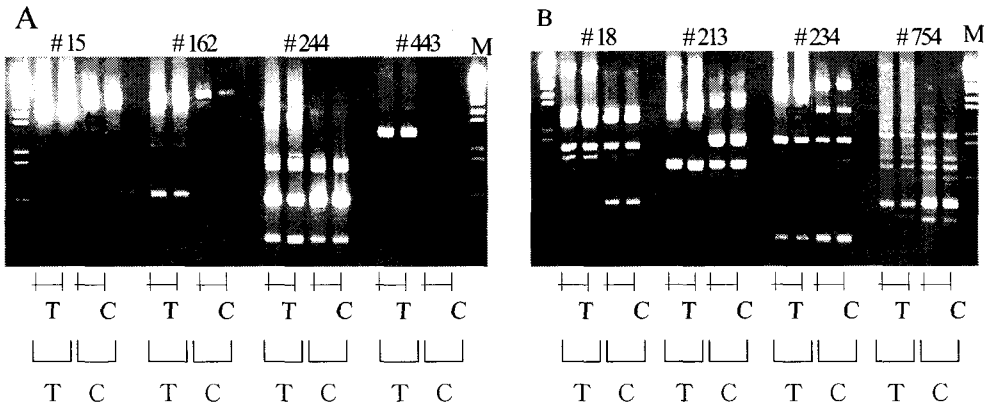

Fig. 1. RAPD assay of horse breed-specific band using pooled genomic DNA.
A) Random primer UBC 15, UBC 162, UBC 244 and UBC 443 showed the Thoroughbred-specific RAPD band patterns 1600 bp, 550 bp, 1700 bp and 1100 bp, respectively.
B) Cheju-native horse specific RAPD fingerprint showed in the random primer UBC 18400bp, UBC 213 850bp, UBC 234 1300bp and UBC 754 350bp, respectively. T : Thoroughbred, C : Cheju-native horse, M : Lamda / *Eco*RI+*Hind* III.

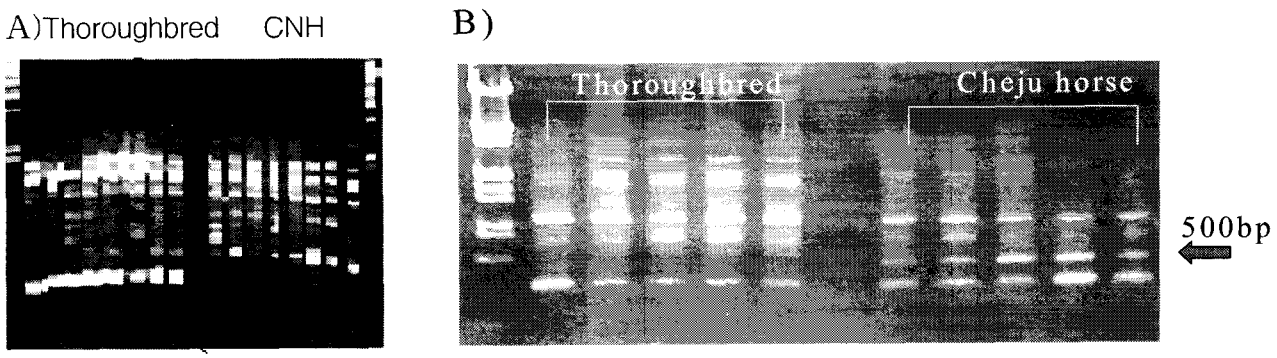

Fig. 2. RAPD analysis of breed specific fragment by individuals.
A) Thoroughbred specific fragment, 550 bp by primer UBC162(A), present in 8 individuals and absent in Cheju-horse.
B) Cheju-horse specific fragment, 500 bp by primer UBC562(B) only present in Cheju-horse.

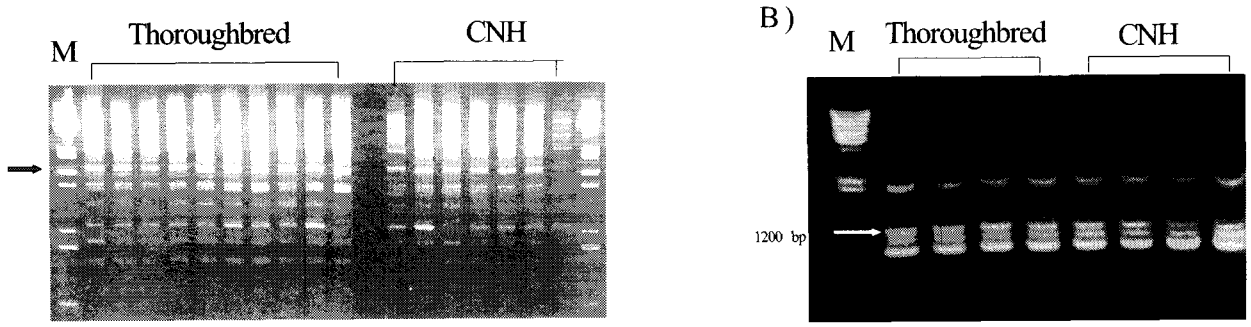

Fig. 3. Comparison of Thoroughbred specific RAPD fingerprint of UBC 85(A) and UBC 126(B)[1]. UBC 851500bp RAPD fingerprint was detected whole Thoroughbred and Cheju-native horse and UBC1261000bp also present Thoroughbred and Cheju-native horse A, B) M : λ/*Hind* Ⅲ, CNH; Cheju-native horse.

promer, we cloned and sequened the breed-specific fragment was shown in Fig. 4. There is no homology with known genes in animals. but it shower the higher homology to rice genome genes. On the basis of this experiment, genetic characteristic of Cheju-native horse showed the very close similarty to Thoroughbred when we compared the band sharing pattern using 800 random primers But we certified the possiblity to make the breed-specific marker between Thorowghbred and Cheju-native horse using RAPD analysis.

A) Thoroughbred specific fragment sequence by UBC 162

*AACTTACCGC*TGCCTTAAGAAAGTATTAAAGAAAAGACAGGAAGAAAGAAAAAAAGCAGAAAGTTTACTACTGAGGAAG
ATAAGAATCATACTAAATATGACAAATTCATCAAATTAGCACTTACAGAAAACAGAAAATTTACAAAATACTACTTTATAG
AGTTAAAAAAAAAAAAAAAAGGACTTGGGACAGCCTACAGACGGAGAGGAGACTCACCTGTCCGAAGAAGGCCACTCGGA
AGTACGTCCCCAAGAGCCTGCGGCCCGAGTGCATGACCTCGGTCACTTTGCTGTAGGCCCGGTGTAGGGTGTCATAGAGAT
GAGCCAGCCTCTGAAAACAGAACAAAACCCATTTCCTCCCACTTGCAACTAGTAGAATCAATTTCAAAATAACAATTAAAT
GAGATAATGTAGGTGAAAATTCCTTGGACTTGAGTTAAAAAAAAGAAAAGATCAAGAATGTATTTTGTCTGTACAACTGGT
AGAGATCACTTCTGAATTGTAATGTTTCCTACCCAAAGGGCCATCTTGCGTATTGAGGACCTATCAGATTTCCATAAA*GCGG*
TAAGTT

B) Cheju-native horse specific fragment sequence by UBC 562

*CAAAGTAGCC*AGGCTGGGAAGCAGATCATCTAATGTGAGTGTGACCCACGTTGGCCACACTGTTGAGAAGGCTCAGGCAGG
GTGGGAAAGAGAAATACAGCAGAAAGAGCATAGGCACTCTATATAAATATATTGAGGACTTTTATTGAAAATCACTTAAA
ATCACAAATGGAGACCCAATATCCATTGCACAACTATGCACGGAAAGCTAAAAAAAAGCTAAAGAAGGCAAGGAAATAGG
CAGAAATTTGAGGTAATCACTGAGCAGCCCTAGGTTTTGCTGGCTAAAGAGTAAGTGAGTTTAGGGGGCAAGCATAGGAA
CCAGTTGGTAAAGATAATTTCTCACTGGCTTCCCTGAATTCATGGGTTCAGGGCTGTATAACCCAACTTGTTATGAAGAAGA
TTGGGAGGAATCTGATAATAAAAGTCATAGTATCACATTAAGTTAAGATCTAGGCAGAGTTAGTTTTAAGAAGTCTATGTC
CGAAATAGTTGACTCTTGAAACACATACAG*GGCTACTTTG*

Fig. 4. The nucleotide sequence of cloned breed-specific fragment of UBC162(A) and UBC 562(B), respectively.

On the basis of this experiment, genetic characteristics of Cheju-native horse showed the very close similarity to Thoroughbred when we compared the band sharing pattern using 800 random primers. We found 9 primers which identified markers present in the pooled DNA from breed but absent in the other breed. Testing individual horse revealed that only 4 marker were wholly absent in breed while present in the other breed. Carrying out the PCR using SCAR-primers didn't show significantly higher specific band pattern. This results suggested that it should be carried out to know the sequence of 5'-flanking region to increase the repeatability of RAPD assay.

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초록 : PCR-RAPD를 이용한 제주말의 유전적 다양성분석

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본 연구는 short oligonucleotide primer를 이용하여 마품종간 유전 분석을 실시하고자 PCR증폭 기법을 확립하고, 확립된 기술을 이용하여 제주도에 사육중인 천념기념물 347호로 등록된 제주말과 경주마로 잘 알려진 더러브렛간의 유전적인 다양성을 분석한 결과마품종간 차이를 보이는 DNA marker는 9개의 primer에서 확인 되었으며, 이중 6개의 primer에서 더러브렛 특이 밴드와 나머지 3개에서 제주마 특이 RAPD 밴드가 확인되어 cloning과 sequencing후에 SCAR primer을 제작하여 마품종 식별에 활용할 수 있을 것으로 사료되며, 본 연구결과 RAPD표지인자는 마품종간의 유전 분석에 매우 유용한 것으로 판단되었다.