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PCR Analysis of Four Length-Polymorphic Loci in Korean Population for Genotyping

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Abstract On human chromosomes, a short sequence of DNA is known to repeat a number of times. These repeats are called variable number of tandem repeat (VNTR) or short tandem repeat (STR) which has a short repeat core. VNTR and STR are used in the field of forensic science, evolution, and anthropology In this work, we examined allele frequencies of one VNTR (YNZ22) and three STRs (NeuR, D21S11, Humth01) in a Korean population sample by polymerase chain reaction (PCR) followed by high-resolution polyacrylamide gel electrophoresis (PAGE) with silver stain. Subsequently, the polymorphism information content (PIC) was calculated . the highest PIC was observed in the NeuR locus (0.95680) and lowest in the Humth01 locus (0.75809)

Keywords: variable number of tandem repeat (VNTR), short tandem repeat (STR), polymorphism information content (PIC), polymerase chain reaction (PCR)

INTRODUCTION

Identity tests, as performed in the fields of paternity testing and forensics, rely on the detection of genetic differences among individuals. The analysis of VNTR and STR loci is a reliable method for human identification, parentage testing and genetic mapping. Typically, tetra nucleotide repeat loci are the choice for most applications because of their high degree of polymorphism in human populations [1]. Genotyping with VNTR and STR loci involves PCR amplification of human genomic DNA, and separation and size determination of the PCR products. This PCR based typing technique quickly became popular because they are less time consuming and yield interpretable results more easily for forensic identification and for determining relatedness of individuals [2]. Slab gel electrophoresis is used most to separate PCR fragments. In a slab gel, determining the size of a PCR fragment is done by comparing the migration distance of a DNA fragment to that of a standard size marker run either in the same lane or in an adjacent lane [3]. The procedure resolves alleles of VNTR and STR into discrete entities. By using an inexpensive silver stain for the detection one can obtain a permanent record of the electrophoretic pattern.

This combination of PAGE and silver staining approach offers certain advantages over the restriction fragment length polymorphism (RFLP) analysis of VNTR and STR loci by Southern blotting. the advantages are (1) discrete allele resolution, (2) correct genotyping of single-band VNTR and STR patterns, (3) a nonisotopic assay, and (4) reduced assay time [4]. In this work, we examined allele frequencies of one VNTR (YNZ22) and three STRs (NeuR, D21S11, Humth01) in a Korean population sample by polymerase chain reaction (PCR) followed by high-resolution polyacrylamide gel electrophoresis (PAGE) with silver stain. The objective of this study was to examine the usefulness of the 4 length-polymorphic loci for genotyping of a Korean population.

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MATERIALS AND METHODS

Samples and DNA Extraction

Sixty buccal swab and 40 plucked hair samples were taken from unrelated Koreans. Buccal swabs and plucked hairs were taken in duplicates from each individual and used for DNA extraction using the chelating resin method [5].

PCR Primers and Amplification Condition

The 4 loci, *i.e.* YNZ22, NeuR, D21S11 and Humth01, were amplified using the specific primer sets described in Table 1. The PCR was carried out in 25 μL reaction mixture made up of 1.5 U Taq DNA polymerase(DMS, Korea), 200 μM dNTP (UTP), 10 pmole of each of the primers, 2.5 μL 10× PCR reaction buffer and 10 to 20 ng of template DNA. Amplification was carried out using Progene (Techne, England) PCR system, and PE2400 (Perkin Elmer, USA) system. The following cycling conditions were employed for the amplification of the YNZ22 locus in a sequential manner: 50°C for 5 min,

Table 1. Primer sequences used for 4 length-polymorphic loci

Target locus	Primer sequence	Repeat core size	References
YNZ22	forward 5' ggTCg AAgAg TgAAg TgCAC Ag reverse 5' CCCAC AgTCT TTATT CTTCA gCg	70 bp	GenBank*
Neurotensin receptor gene	forward 5' CATCA gCTCA gAAgC AgATA gT reverse 5' AgAgC AAgAA CTCCA TgTCT AAg	4 bp	GenBank*
D21S11	forward 5' ATgTg AgTCA ATTCC CCAAg TgA reverse 5' gTTgT ATTAg TCAAT gTTCT CCAg	4 bp	GenBank*
Human tyrosine hydroxylase gene	forward 5' TgATT CCCAT TggCC TgTTC CT reverse 5' AgCTC CCgAT TATCC AgCCT g	4 bp	GenBank*

*http://www ncbi nlm nih.gov

95°C for 5 min - UNG (Uracil DNA glycosylase) reaction, initial denaturation; 94°C for 35 sec, 62°C for 35 sec, 72°C for 1 min and 30 sec 30 cycles; and for 3 STR loci (NeuR, D21S11 and Humth01): 50°C for 5 min, 95°C for 5 min - UNG (Uracil DNA glycosylase) reaction, initial denaturation; 94°C for 35 sec, 62°C for 35 sec, 72°C for 35 sec 35 cycles The final extension was done at 72°C for 5 min.

Electrophoresis and Detection of PCR Product

YNZ22 PCR products were separated by 1.5% agarose gel electrophoresis. PCR products amplified from NeuR, D21S11 and Humth01 loci were separated by 5% to 8% high-resolution PAGE prior to being silver-stained as previously described [4].

Statistical Analysis

Polymorphism Information Content (PIC) values were calculated according to the equation below

$$1-(\sum_{i=1}^{n} Pi^2)-\sum_{i=1}^{n-1}\sum_{j=i-1}^{n} 2Pi^2 2Pj^2$$

n : number of alleles, Pi · allele frequencies

Loci with many alleles and a PIC near 1 are most desirable. Such loci will probably be useful for identification of human individuals [6]

RESULTS AND DISCUSSION

Figs. 1, 2 and 3 illustrate that the analysis of four-length polymorphic loci can be successfully performed using the technique described in this paper. Each allele was completely resolved based on the repeat unit increment of the VNTR and STR loci. We obtained the genotype of each locus from the analysis of PCR product length in Korean populations. The distribution of observable genotypes for VNTR and STR loci in the Korean population sample is shown in Table 2 With the VNTR locus (YNZ22), thirteen different alleles

Fig 1. A representative 1 5% agarose gel displaying YNZ22 profiles Samples from left to right are size marker, 6-6, 1-7, 7-7, 4-5, 5-5, 6-6, 5-8, 1-6, 4-4, 2-10, 2-3, 5-5, 4-5, 5-5, 2-15 and 4-6, size marker. The size standards are 1-kb plus DNA ladder (Gibco BRL). The cathode is at the top.

were observed in 77 unrelated Koreans. The alleles were designated from 1 through 15 (allele 9 and 14 were not observed), where allele 1 was the shortest and allele 15 was the longest in length. One advantage of using YNZ22 locus is easiness in genotyping using 1.5% agarose gel since it has the repeat core site of 70 bp. In a previous study, the efficiency of amplification or yield of PCR products was shown to be related to the length of the target site between the primers [7]. For example, at the D17S30 locus, it was observed by Horn *et al.* [7] that larger alleles could be amplified to a significantly less extent than smaller ones. However, all YNZ22 alleles examined to the date are less than 1Kb in length. As shown in Fig. 1, there is no apparent difference in the band intensity between the largest (allele 15) and the smallest alleles (allele 1) Thus, the amplification product length-polymorphism analysis of YNZ22 permits correct genotyping of VNTR profiles. This is in contrast to the situation in RFLP analysis by Southern blotting, where correct genotyping may not always be possible. Larger DNA fragments, which contain a higher number of repeat sequences, are more readily detectable by hybridization assays than smaller fragments. Thus, some small-sized VNTR alleles may go undetected by RFLP analysis, and single-band patterns, detected by RFLP analysis via Southern blotting, may or may not be true homozygotes

Table 2. Distribution of VNTR and STR loci genotypes from Korean population samples

Genotype*	YNZ22[a] No. observed	Genotype*	NeuR[b] No observed	Genotype*	D21S11[c] No. observed	Genotype*	Humth01[d] No observed
1-1..	11	55-65.	1	29-31 .	1	5-5. .	1
1-2...	4	58-66...	1	29-33...	1	5-6...	1
1-3...	2	59-60.	1	29-34 .	1	5-7...	1
1-4...	2	60-76...	1	29-30...	1	5-8...	10
1-5...	1	61-61...	1	30-30...	3	6-6...	3
1-6 .	2	61-68.	1	30-31...	9	6-7...	6
1-7 ..	2	61-74 .	2	30-32 ..	3	6-8..	12
2-2...	1	62-68...	1	30-33...	1	6-9...	11
2-3...	1	62-73...	1	30-34...	2	7-7...	4
2-4...	1	63-67...	1	31-31...	10	7-9...	4
2-10...	1	63-69 ..	1	31-32...	10	8-8...	15
2-12 .	1	63-75..	1	31-33...	3	8-9...	9
2-15...	1	64-64 ..	1	31-34...	10	9-9...	3
3-3...	2	64-68.	2	31-35...	4	9-10...	7
3-4...	3	64-70...	2	31-36...	1	10-10	1
4-4...	6	64-73...	3	32-32...	12		
4-5...	7	64-75...	3	32-33 .	5		
4-6...	1	64-78.	1	32-34...	8		
4-8 .	1	65-65..	3	32-35...	4		
4-11...	1	65-67 ..	1	32-37...	1		
4-13. .	1	65-69...	2	33-33...	2		
5-5.	5	65-70...	1	33-34 .	2		
5-6. .	2	65-71...	2	33-36 ..	2		
5-8...	1	66-66...	1				
5-10 .	1	66-67 ..	1				
5-11...	3	66-68 .	1				
6-6	5	66-69...	4				
6-7..	1	66-70...	1				
6-8...	1	66-72 .	2				
6-10...	1	66-73 ..	2				
6-11...	1	66-74 ..	2				
6-12...	1	66-76...	3				
7-7...	3	66-78...	1				
		67-67...	4				
		67-70 ..	1				
		67-74 ..	2				
		67-75...	1				
		68-68...	1				
		68-70...	2				
		68-73 .	2				
		68-76...	1				
		69-69...	2				
		69-71 .	1				
		69-72...	1				
		69-73...	2				
		70-70...	1				
		70-74. .	1				
		70-75...	2				
		71-72 .	1				
		71-73 ..	2				
		72-72...	1				
		72-74...	2				
		72-75...	1				
		73-73...	2				
		73-75	1				
		73-78 ..	1				
		75-75..	2				
		75-77..	1				
33		58		23		15	

a VNTR locus, b STR locus neurotensin receptor gene, c. STR locus, d. STR locus: human tyrosine hydroxylase

* Genetic composition based on the number of repeated core.

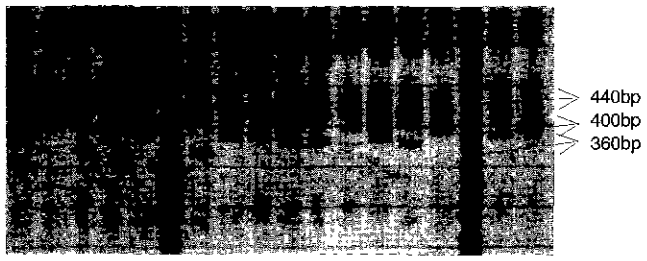

Fig 2. Silver-stained PAGE displaying NeuR profiles. Samples from left to right are 65-70, 73-75, 61-74, 70-75, 70-74, 20 bp ladder, 60-76, 66-70, 66-70, 65-75, 65-75, 65-70, 65-70, 65-75, 68-76, 20 bp ladder, 66-76 and 70-68. The cathode is at the top

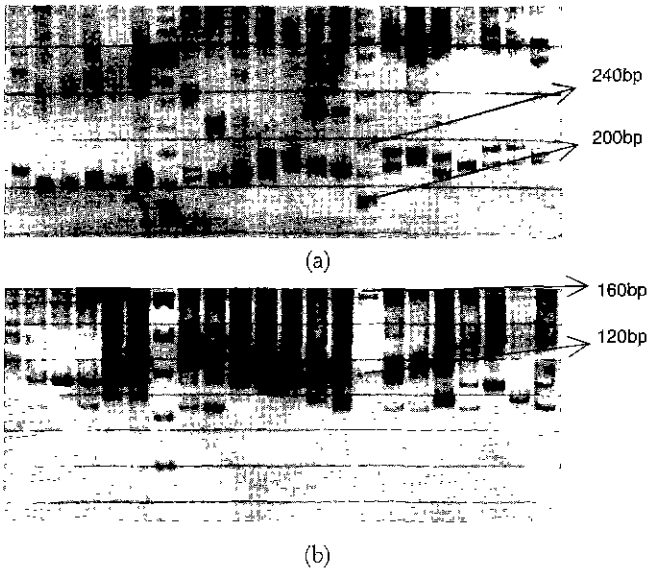

Fig. 3 Silver-stained PAGE displaying D21S11 (a) and Humth01 (b) profiles. (a) Samples from left to right are 33-33, 30-31, 31-31, 32-32, 31-32, 31-31, 20 bp ladder, 31-33, 30-32, 32-32, 32-34, 33-34, 32-33, 32-32, 20 bp ladder, 32-34, 33-34, 31-33, 32-32, 32-34, 32-34, and 32-33. (b) Samples from left to right are 6-7, 7-9, 9-9, 6-9, 7-9, 7-9, 20 bp ladder, 6-9, 6-9, 9-10, 9-10, 9-10, 7-9, 6-7, 20 bp ladder, 6-9, 6-6, 7-7, 6-9, 9-9, 7-7, and 6-9. The size standards are 20 bp DNA ladder (Gibco BRL). The cathode is at the top.

Neurotensin Receptor gene: Twenty two different alleles were observed in 90 unrelated Koreans. The alleles were designated from 55 through 78 (allele 56 and 57 were not observed), where allele 55 was the shortest and allele 78 was the longest in length.

D21S11: Nine different alleles were observed in 96 unrelated Koreans The alleles were designated from 29 through 37, where allele 29 was the shortest and allele 37 was the longest in length. In Japanese populations, these latter alleles were not observed [8]. In the D21S11 locus, comparison of the alleles between Korean and Japanese populations showed significant differences. Thus, D21S11 appears to be a useful locus to distinguish between a Korean and a Japanese.

Human tyrosine hydroxylase gene: Six different alleles were observed in 88 unrelated Koreans. The alleles

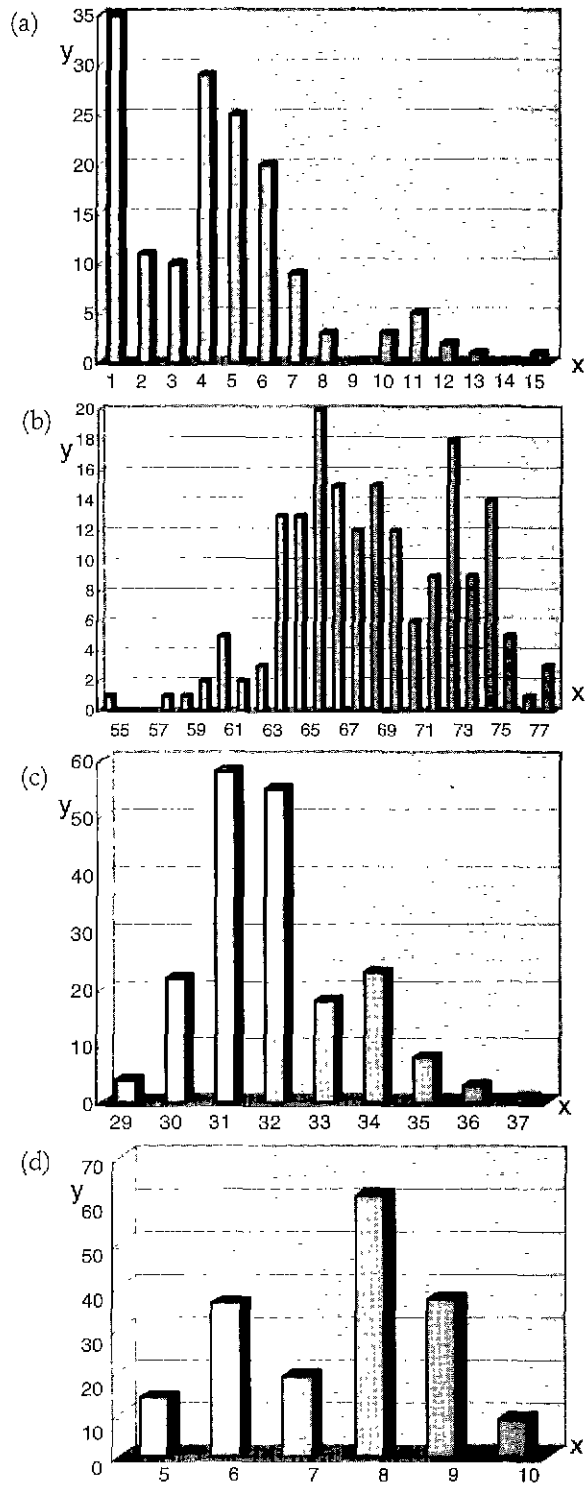

Fig. 4. Alleles distribution of four length-polymorphic loci in Korean population. (a) YNZ22, (b) Neurotensin receptor gene, (c) D21S11, (d) Human tyrosine hydroxylase gene. x: alleles y: number observed.

were designated from 5 through 10, where allele 5 was the shortest and allele 10 was the longest in length. In

Table 3. Allele frequencies, polymorphism information content from analyzed VNTR and STR loci from unrelated Korean population

Allele	YNZ22*	Allele	NeuR*	Allele	D21S11*	Allele	Humth01*
1	0 22727	55	0.00556	29	0.02083	5	0.07955
2	0.07143	56	0.00000	30	0.11458	6	0.20454
3	0.06494	57	0.00000	31	0.30208	7	0.10795
4	0.18831	58	0.00556	32	0.28646	8	0.34659
5	0 16234	59	0.00556	33	0.09375	9	0.21023
6	0 12987	60	0 01111	34	0.11979	10	0 05114
7	0.05844	61	0 02778	35	0.04167		
8	0.01948	62	0.01111	36	0.01563		
9	0.00000	63	0.01667	37	0.00521		
10	0.01948	64	0.07222				
11	0.03247	65	0 07222				
12	0 01299	66	0.11111				
13	0 00649	67	0.08333				
14	0.00000	68	0.06667				
15	0.00649	69	0 08333				
		70	0.06667				
		71	0.03333				
		72	0 05000				
		73	0.10000				
		74	0.05000				
		75	0.07778				
		76	0.02778				
		77	0.00556				
		78	0 01667				
PIC#	0.85112		0.95680		0.76885		0.75809

* The values represent the frequency of each allele among the population examined

\# PIC value was calculated by the formula in the Materials and Methods section.

the Human tyrosine hydroxylase gene, the distribution of alleles in Koreans is very similar to that in Japanese except the presence of allele 11 in Japanese populations [8]. In African-American populations, the alleles were designated from 6 through 10 [9]. Fig. 4 shows the allele distribution of the four length- polymorphic loci in Korean population The allelic frequencies based on the allele distribution for VNTR and STR loci in Korean population samples is shown in Table 3. In Korean populations, the most frequent alleles appear to be allele 1 (f=0.22727) of YNZ22, allele 66 (f=0.11111) of NeuR, allele 31 (f=0.30208) of D21S11, and allele 9 (f= 0.21023) of Humth01. Although all four loci we examined were highly informative (PIC>0.5), the highest PIC was observed for the NeuR locus (0.95680) and lowest for the Humth01 locus (0.75809) among the four loci examined, indicating that the NeuR locus may be the most useful locus for genetic identification of Korean population.

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REFERENCES

[1] Gao, Q., H. M Pang, and E. S. Yeung (1999) Simultaneous genetic typing from multiple short tandem repeat loci using a 96-capillary array electrophoresis system. *Electrophoresis* 20: 1518-1526

[2] Chakraborty, R., D. N. Stivers, B. Su, Y. Zhong, and B. Budowle (1999) The utility of short tandem repeat loci beyond human indentification: Implications for development of new DNA typing systems. *Electrophoresis* 20: 1682-1696.

[3] Marino, M. A., J. M. Devaney, P. A. David, J. K. Smith, and J. E. Girard (1998) Spectral measurements of intercalated PCR-amplified short tandem repeat alleles. *Anal. Chem.* 70: 4514-4519.

[4] Budowle, B., R. Chakraborty, A. M. Ginsti, A. J. Eisenberg, and R. C Allen (1991) Analysis of the VNTR locus D1S80 by the PCR followed by high-resolution PAGE. *Am. J. Hum. Genet.* 48: 137-144.

[5] Silva, F., L. Gusmao, C. Alves, R. Seruca, L. David, and A. Amorim (1997) Tetra- and pentanucleotide short tandem repeat instability in gastric cancer. *Electrophoresis* 18: 1633-1636.

[6] Botstein, D., R. L. White, M. Skolnick, and R. W Davis (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms *Am J Hum. Genet.* 32: 314-331.

[7] Horn, G. T , B. Richards, and K W Klinger (1989) Amplification of a highly polymorphic VNTR segment by the polymerase chain reaction. *Nuc. Acids Res.* 17: 2140.

[8] Hashiyada, M (2000) Short tandem repeat analysis in Japanese population. *Electrophoresis* 21: 347-350.

[9] Robertson, J. M., J. B. Sgueglia, C. A. Badger, A. C. Juston, and J. Ballantyne (1995) Forensic applications of a rapid, sensitive, and precise multiplex analysis of the four short tandem repeat loci Humvwf31/A, Humth01, Humf13a1, and Humfes/fps. *Electrophoresis* 16: 1568-1576.

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