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PCR-based genotyping of Korean population for forensic applications

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ABSTRACT

In human chromosome, a short sequence of DNA has been repeated a number of times. These repeats are called variable number of tandem repeat(VNTR) or short tandem repeat(STR) which has 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 3 VNTR(YNZ22, NeuR, D21S11) and one STR(Humth01) in a Korean population sample by polymerase chain reaction(PCR) followed by high-resolution polyacrylamide gelelectrophoresis(PAGE) with silver staining. Subsequently, the polymorphism information content(PIC) was calculated : the highest PIC was observed for the NeuR locus(0.95680) and lowest for the Humth01 locus(0.75809).

Keyword : Variable Number of Tandem Repeat(VNTR) / Short Tandem Repeat(STR) / Polymorphism Information Content(PIC) / Polymerase Chain Reaction(PCR)

INTRODUCTION

The analysis of VNTR and STR loci is 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.[7] Genotyping with VNTR and STR loci involves PCR amplification of human genomic DNA, separation and size determination of the PCR products. This PCR based typing techniques quickly became popular because they are less time-consuming and yield more easily interpretable results for forensic indentification and for determining relatedness of individuals.[4] The separation of the PCR fragments is mostly performed by slab gel electrophoresis. In slab gel, the size determination of PCR fragments can be performed 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.[7] The procedure resolves alleles of VNTR and STR into discrete entities. By using an inexpensive silver stain for detection one can obtain a permanent record of the electrophoretic pattern. This combination of PAGE and silver staining approach offers certain advantages over the RFLP analysis of VNTR and STR loci by Southern blotting : (1) discrete allele resolution, (2) correct genotyping of single-band VNTR, STR patterns, (3) a nonisotopic assay, and (4) reduced assay time.[2] The objective of this

study was to examine the usefulness of 4 polymorphic loci in forensic applications for a Korean population.

MATERIALS AND METHOD

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 duplicate from each individuals and used for DNA extraction using the chelating resin method.[6]

PCR primers and amplification condition

The 4 loci, YNZ22, NeuR, D21S11 and Humth01, were amplified using specific primer sets described in Table 1. The PCR was carried out in 25ul reaction mixture made up of 1.5ul Taq DNA polymerase, 200uM dNTP(UTP), 10pmole of each of the primers, 2.5ul 10× PCR reaction buffer and 5ul of template DNA.

TABLE 1. Primer sequences used for 4 length polymorphic loci

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

Electrophoresis and detection of PCR product

YNZ22 PCR product was 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.

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 : 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[8].

RESULTS AND DISCUSSION

Analysis of four length polymorphic loci from Korean population

Figures 1 and 2 show that analysis of four length polymophic loci can be performed using the techniques described in this paper. YNZ22 : Thirteen different alleles were observed in 77 unrelated Koreans. The alleles have been designated 1-15(allele 9 and 4 are not observed), where allele 1 is the smallest and alleles 17 is the largest in length. Neurotensin Receptor gene : Twenty two different alleles were observed in 90 unrelated Koreans. The alleles have been designated 55-78(allele 56 and 57 are not observed), where allele 55 is the smallest and allele 78 is the largest in length. D21S11 : Nine different alleles were observed in 96 unrelated Koreans. The alleles have been designated 29-37, where allele 29 is the smallest and allele 37 is the largest in length. Human tyrosine hydroxylase gene : Six different alleles were observed in 88 unrelated Koreans. The alleles have been designated 5-10, where allele 5 is the smallest and allele 10 is the largest in length.

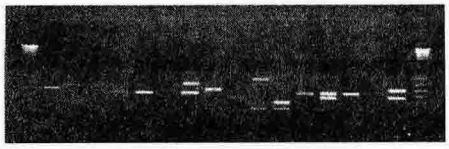

Figure 1. Agarose gel displaying YNZ22 profiles. The YNZ22 types from left to right are 6-6, 1-7, 7-7, 4-5, 5-5, 6-6, 5-8, 1-6, 4-4, 2-1, 2-3, 5-5, 4-5, 5-5, 2-15 and 4-6. The size standards are 1-kb plus DNA ladder(Gibco BRL). The cathode is at the top.

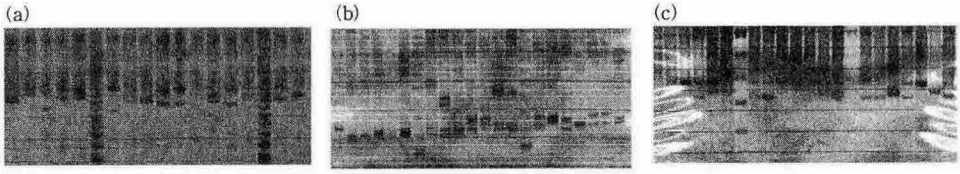

Figure 2. Siver-stained PAGE displaying 3 STR loci profiles. (a) The NeuR types from left to right are 65-70, 73-75, 61-74, 70-75, 70-74, 60-76, 66-70, 66-70, 65-75, 65-75, 65-70, 65-70, 65-75, 68-76, 66-76 and 70-68. (b) The D21S11 types from left to right are 33-33, 30-31, 31-31, 32-32, 31-32, 31-31, 31-33, 30-32, 32-32, 32-34, 33-34, 32-33, 32-32, 32-34, 33-34, 31-33, 32-32, 32-34, 32-34, and 32-33. (c) The D21S11 types from left to right are 6-7, 7-9, 9-9, 6-9, 7-9, 7-9, 6-9, 6-9, 9-10, 9-10, 9-10, 7-9, 6-7, 6-9, 6-6, 7-7, 6-9, 9-9, 7-7, and 6-9. The size standards are 20bp DNA ladder(Gibco BRL). The cathode is at the top.

Allele frequencies and polymorhism information content

The allelic frequencies for VNTR and STR loci in Korean population sample are shown in Table 2. In our population the most frequent alleles are allele 1(f=0.22727) of the YNZ22, allele 66(f=0.11111) of NeuR, allele 31(f=0.30208) of D21S11, allele 9(f=0.21023) of Humth01 indicating that examined four loci are highly informative(PIC>.5). Among the four loci examined the highest PIC was observed for the NeuR locus and lowest for the Humth01 locus(Table2), indicating that NeuR locus is most useful locus in forensic analyses

Table 2. Allele frequencies, polymorphism information content for analyzed VNTR, 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

ACKNOWLEDGMENTS

This work was supported by the Brain Korea 21 Project.

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