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DNA Polymorphism Analysis of the HLA-DRB1 Gene Using Polymerase Chain Reaction-Sequence Specific Primer (PCR-SSP) among Korean Subjects

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Abstract: Most expressed HLA loci exhibit a remarkable degree of allelic polymorphism, which derives from sequence differences predominantly localized to discrete hypervariable regions of the amino-terminal domain of the molecule. In this study, the HLA-DRB1 genotypes were determined in eighteen control cell lines and 112 unrelated Koreans using the PCR-SSP (Polymerase Chain Reaction-Sequence Specific Primer) technique. 29 specific primer pairs in assigning the DRB1 gene were used. The results of control cells correlated well with the data which was previously reported. The heterozygosity and homozygosity of the DRB1 gene were 0.786 and 0.214, respectively. In a total of 41 different DRB1 alleles and 83 genotypes, the most frequent allele and genotype were DRB1*04 and DRB1*0901/1501, respectively. This study shows that the PCR-SSP technique is relatively simple, fast and a practical tool for the determination of the HLA-DRB1 genotypes. Moreover, these results-allele and genotype frequency and heterozygosity of the HLA DRB1 gene-could be useful for database study before being applied to individual identification and transplantation immunity.
Key words: genotyping, HLA-DRB1, PCR-Sequence Specific Primer.

The polymorphisms of HLA Class II genes (DR, DP, DQ) are confined to the membrane-distal domain, encoded by the second exon of the respective gene (Inoko, 1991). These highly polymorphic HLA molecules bind to foreign or self antigenic peptides and present them to antigen-specific T cells in a self-restricted fashion and specify heterodimeric glycoproteins involved in the regulation of the immune response (Schwartz, 1985; Bodmer *et al.*, 1994). In the elucidation of HLA polymorphism, serological and cellular typing methods have been replaced by genetic typing using PCR (Polymerase Chain Reaction) (Randall *et al.*, 1989; Thonnard *et al.*, 1995). Accurate identification of HLA polymorphisms is a prerequisite for determination of the functional role of HLA genes. However small genetic differences among HLA Class II genes make it difficult to do accurate HLA genotyping. Recently several genotyping methods for HLA Class II genes, PCR-SSO (Schart *et al.*, 1991), a nonradioactive reverse dot blot (Bugawan *et al.*, 1990), PCR-SSCP (Single Strand Conformation Polymorphism) (Hoshino, 1992), PCR-RFLP (Restriction Fragment Length Polymorphism) (Nomura *et al.*, 1991; Lee and Park, 1994, 1995; Lee, 1994, 1995), PCR-SSP (Sequence Specific Primers) (Park and Tonai, 1992; Olerup *et al.*, 1993; Chia *et al.*, 1994) and the direct sequencing method (Santamaria *et al.*, 1992) have been introduced. In this study, PCR-SSP was used for the detection of the polymorphism of the HLA-DRB 1 gene. The PCR-SSP technique was first described for the diagnosis of sickle-cell anemia (Saiki *et al.*, 1985), α_1-antitrypsin deficiency (Newton *et al.*, 1989), cystic fibrosis (Ballabio *et al.*, 1990) and apolipoprotein (Schuster *et al.*, 1992). For the HLA-DRB1 genotyping using PCR-SSP, the 29 specific primer pairs were designed for identifying allelic variability corresponding to the serologically defined specificities. The absence or presence an amplified product has mostly been detected by agarose gel electrophoresis. After that, the patterns of DNA fragments were systematically investigated to test whether they were correlated with HLA DR specificities. From these results, it can be said that the PCR-SSP technique for HLA-DRB1 genotyping is a simple, rapid and accurate technique at the nucleotide level, and can be substituted for serological and cellular typing in routine work. Also high heterozygosity and

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polymorphic alleles of the HLA-DRB1 gene show the usefulness of the clinical application among Koreans.

Materials and Methods

Study subjects and cell lines

One hundred twelve randomly selected healthy Korean individuals from Seoul Medical Science Institute were investigated. For the control of the experiment, eighteen homozygous control cell lines from the Xth International Histocompatibility Workshop were used. They were analyzed by the southern blot method (Maeda and Murayama, 1989).

Synthesis of sequence specific primers

Primers were designed using the nucleic acid sequence of exon 2 of the HLA-DRB1 gene published by Marsh and Bodmer (1992). Amplification primers were synthesized using a 392 DNA/RNA synthesizer and purified by an oligonucleotide purification cartridge (Applied Biosystem, USA). The specificity of each individual primer synthesis was tested against eighteen homozygous cell lines. Table 1 shows the sequences of 29 specific primer pairs and the sizes of PCR products for DRB1 PCR-SSP typing.

Extraction and amplification of DNA

High molecular weight DNA was isolated from proteinase-K-treated peripheral blood leukocytes by phenol/chloroform extractions and ethanol precipitation (Guistincich *et al.*, 1991). For HLA-DRB1 DNA typing by PCR-SSP, 13 separate PCR primer mixtures containing two to three sequence specific DRB1 primer pairs were performed per sample. Primer mixtures, containing all the ingredients of the PCR reaction except DNA and *Taq* polymerase, were prepared in 500 μl batches sufficient for 60 typings. 8 μl primer mix was added to 1 μl of DNA (1 μg) and 1 μl of diluted AmpliTaq (0.25 units in 1×PCR buffer). The primer mixtures contained 0.4 μM of the allele specific primers, 56 mM KCl, 1.7 mM $MgCl_2$, 11 mM Tris HCl, pH 8.3, 0.0011% (w/v) gelatin and 250 μM each of dNTPs. Primer mixtures were kept at +4℃ for up to a month and stored for longer periods at −20℃. Thirty amplification cycles were carried out in an automated thermal cycler (GeneAmp PCR system 9600. Perkin Elmer Cetus Inc.). Each cycle consisted of denaturation at 94℃ for 20 seconds, annealing at 59℃ for 50 seconds and extension at 72℃ for 20 seconds. Primers without DNA were used as a negative control.

Visualization of amplified DNA and statistical evaluation

PCR products were visualized by agarose gel electrophoresis (Bio Rad Co. Ltd.). After the addition of 2 μl of loading buffer (30% (v/v) glycerol stained with bromophenol blue and xylene cyanol), the PCR products were loaded in 2% (w/v) agarose gels pre-stained with ethidium bromide (0.5 μg/ml gel). Gels (8.5×10 cm) were run for 15 min at 10 volt/cm in 0.5×TBE buffer (89 mM Tris base/89 mM boric acid/2mM EDTA, pH 8.0) without buffer re-circulation (Southern, 1975). Gels were examined under UV illumination and documented by photography (Markovits *et al.*, 1979). Allele size was analyzed using the Gene Scanner (Applied Biosystems, USA). The degree of individual specificity of genotypes determined by HLA-DR alleles can be estimated from the heterozygosity H. The mean allele frequency q at a locus is given by (1−H), and assuming that all alleles share the same population frequency q, then the probability that two randomly selected 2 unrelated individuals possessing the same genotype can be estimated as $q^2(2-q)$ (Lee, 1994). The frequency of each allele in the population was calculated from the numbers of each genotype that were collected in the sample set.

Results

All eighteen control cell lines and 112 individuals were able to be matched by DRB1 PCR-SSP typing. No false-positive amplifications were observed in the 24 homozygous and 88 DRB1 heterozygous individuals and cell lines investigated. The genotypes of eighteen control cells analyzed by PCR-SSP correlated well with data previously reported (Table 2) (Maeda and Murayama, 1989; Kimura *et al.*, 1992).

The allele and genotype frequencies in the amplified HLA-DRB1 gene in the Korean population

Twenty nine primer sequences and the DNA fragment sizes for DRB1 genes are shown in Table 1. Table 3 shows the allele frequencies of the HLA-DRB1 gene in 112 unrelated Koreans using PCR-SSP. A total of 41 different alleles and 83 genotypes were observed by 13 PCR primer mixures. The HLA-DRB1*04 group was most commonly observed and HLA-DRB1 alleles occurred at frequencies of 0.004−0.121 (Table 3). In 83 DRB1 genotypes, DRB1*0405 (homozygote, f=0.027) and DRB1*0901/1501 (heterozygote, f=0.045) were frequently observed (Table 4). The observed heterozygosity and homozygosity of the HLA-DRB1 gene was 0.786 and 0.214, respectively (Table 4). The amplified DNA fragments of DRB1 ranged between 111 and 270 bps in length (Table 1). Fig. 1 and 2 show the amplified DNA fragments of the DRB1*

Table 1. The sequences of 29 DRB1 specific primer pairs and PCR products for HLA-DRB1 typing

Group	HLA DRB1 alleles	PCR		
		Products (bps)	(5′ primer)	(3′ primer)
1	DRB1*0101	219	GCGGGTGCGGTTGCTGGAA	TGCACTGTGAAGCTCTCAC
2	DRB1*0102	219	GCGGGTGCGGTTGCTGGAA	TGCACTGTGAAGCTCTCCA
	DRB1*0101,0103	169	GCGGGTGCGGTTGCTGGAA	GTCCACCGCGGCCCGCTC
3	DRB1*1501	255	TCCTGTGGCAGCCTAAGAGG	TGCACTGTGAAGCTCTCCA
	DRB1*1501,1502	213	TCCTGTGGCAGCCTAAGAGG	TCCACCGCGGCCCGCGC
4	DRB1*1601	201	TCCTGTGGCAGCCTAAGAGG	CGCGCCTGTCTTCCAGGAA
	DRB1*1502,1601,1602	258	TCCTGTGGCAGCCTAAGAGG	TGCACTGTGAAGCTCTCAC
5	DRB1*0301,0302,1301,1302,1305,1402,1403	111	GTTTCTTGGAGTACTCTACGTC	CGCTGTCGAAGCGCACGTT
	DRB1*1101,1102,1103,1104,1105	174	GTTTCTTGGAGTACTCTACGTC	CAGGCTGTTCCAGTACTCCT
	DRB1*1101,1103,1104	191	GTTTCTTGGAGTACTCTACGTC	GCGCCTGTCTTCCAGGAA
	DRB1*0301,0302	222	GTTTCTTGGAGTACTCTACGTC	TGCAGTAGTTGTCCACCCG
	DRB1*0301,1102,1103,1104,1301,1304,1401	261	GTTTCTTGGAGTACTCTACGTC	CTGCACTGTGAAGCTCTCCA
6	DRB1*0302,1101,1105,1302,1303,1305,1403	259	GTTTCTTGGAGTACTCTACGTC	CTGCACTGTGAAGCTCTCAC
	DRB1*1102,1103,1105,1301,1302,1304	210	GTTTCTTGGAGTACTCTACGTC	GTCCACCGCGGCCCGCTC
	DRB1*1303,1304	171	GTTTCTTGGAGTACTCTACGTC	TGTTCCAGTACTCGGCGCT
	DRB1*1401,1405	117	GTTTCTTGGAGTACTCTACGTC	CGCTGTCGAAGCGCACGAA
7	DRB1*0406	111	GTTTCTTGGAGCAGGTTAAACA	GCTGTCGAAGCGCACGG
	DRB1*0405,0409,0410,0411	171	GTTTCTTGGAGCAGGTTAAACA	TGTTCCAGTACTCGGCGCT
	DRB1*0403,0406,0407	222	GTTTCTTGGAGCAGGTTAAACA	TCTGCAGTAGGTGTCCACCT
	DRB1*0401,0405,0407,0408,0409	261	GTTTCTTGGAGCAGGTTAAACA	CTGCACTGTGAAGCTCTCAC
8	DRB1*0402	214	GTTTCTTGGAGCAGGTTAAACA	GTCAACCGCGGCCCGCTC
	DRB1*0402,0403,0404,0406	261	GTTTCTTGGAGCAGGTTAAACA	CTGCACTGTGAAGCTCTCCA
9	DRB1*0801,0803	165	TTTCTTGGAGTACTCTACGGG	TGTTCCAGTACTCGGCGCT
	DRB1*1201,1202,1404	210	TTTCTTGGAGTACTCTACGGG	CCACCTCGGCCCGCCTCC
	DRB1*0804,1201,1202,1404	261	TTTCTTGGAGTACTCTACGGG	CTGCACTGTGAAGCTCTCCA
10	DRB1*0801,0802,1202	261	TTTCTTGGAGTACTCTACGGG	CGCGCCTGTCTTCCAGGAA
11	DRB1*0701	267	TCCTGTGGCAGGGTAAGTAT	CGCTGCACTGTGAAGCTCTC
12	DRB1*0901	159	GAGCGGGTGCGGTATCTGC	CCACCTCGGCCCGCCTCC
13	DRB1*1001	159	GCGGGTGCGGTTGCTGGAA	CCACCGCGGCACGCCTCC

0901 control cell (Cell Number: 9075, Cell Name: DKB) and DRB1*0803 control cell (Cell Number: 9070, Cell Name: LUY), respectively. Examples of HLA-DRB1 alleles by PCR-SSP are shown in Fig. 3 (DRB1*0803/*1302) and Fig. 4 (DRB1*0802/*0901).

Discussion

Until the last few years, HLA Class II allelic polymorphism has been identified with serological, cellular and biochemical techniques (Carlson *et al.*, 1987). However, DNA typing methods are becoming more widely used as an alternative to serological typing (Erlich *et al.*, 1989; Helga *et al.*, 1995). The extensive polymorphism of the HLA-DRB1 locus, as in -DQ and -DP genes, is confined to the membrane-distal domain encoded by the second exon. In general, the pattern of second-exon allelic variablility is a patchwork of specific polymorphic segments found in several different alleles (Schart *et al.*, 1991). Further allelic differences of the HLA-DRB1 gene between donor and recipient in cases of organ transplantation sometimes cause a high incidence of complications (Beatty *et al.*, 1991). Therefore a simple and accurate genotyping of the HLA-DR region is important for clinical work. The flanking sequences of 2 exon of DRB1 are locus-specific and highly conserved between alleles. This enables group-specific amplifications preceding allelic analysis using sequence-specific primers in PCR-SSP (Olerup *et al.*, 1993). The PCR product may be used for genomic typing, directly identifying expressed genetic variability (Tiercy *et al.*, 1990; Tiercy and Friedel, 1991). In this experiment, the PCR-SSP method, which is a powerful technique for detecting genetic variability with a high degree of resolution, was used for HLA-DRB1 DNA typing (Park

Table 2. The genotypes of 18 control cell lines using PCR-SSP

WSNo	Cell line	DRB1
9005	HOM2	DRB1*0101
9002	MZ70782	DRB1*0102
9036	SP0010	DRB1*1101
9008	D0208915	DRB1*1501
9010	AMAI	DRB1*1503
9017	WT8	DRB1*1501
9058	OMW	DRB1*1301
9062	WDV	DRB1*1301
9050	MOU	DRB1*07**
9047	PLH	DRB1*07**
9075	DKB	DRB1*0901
9029	WT5	DRB1*0401
9068	BM9	DRB1*0801
9021	RSH	DRB1*0302
9018	L0081785	DRB1*0301
9019	DUCAF	DRB1*0301
9023	VAVY	DRB1*0301
9070	LUY	DRB1*0803

and Tonai, 1992; Chia *et al.*, 1993). Primers were designed for the PCR cycle profile and the compositions of the 13 PCR reaction mixtures were adjusted to obtain highly specific and sensitive amplifications for the assignment of all phenotypically expressed DRB1 polymorphism. The melting temperature (Tm) of the primer was adjusted to be kept 3~5℃ below the annealing temperature of the PCR cycle to achieve specific, efficient and reproducible allele-specific amplification. Tm was maintained between 55℃ and 57℃. The allele frequencies of the DRB1 gene were compared with those in a previous report determined by the two step PCR method (Han *et al.*, 1992). The most frequent allele group of HLA-DRB1 gene was DRB1*04 in both studies (Table 3). The HLA-DRB1 gene was hypervariable and polymorphic because of its high heterozygosity (0.786) and 41 different alleles (Table 4). In population genetic studies, the expected number of genotypes as well as the observed number of genotypes were based on the assumption of Hardy-Weinberg expectations (Nei and Roychoudhury, 1974). In this experiment, however, the expected number of genotype frequencies of the DRB1 gene was not observed because the Chi-Square may not be a valid test when the minimal expected allele number(n) is less than 5 (Kloosterman *et al.*, 1993). It is desirable to repeat this study in a larger group for statistical value. The authors previously reported HLA-DQA1 and DQB1 genotyping using PCR-RFLP (Lee and Park, 1994; Lee, 1995). This method, however, had several problems in the typing of highly hypervariable genes. First, partial digestion of the restriction enzyme was frequently observed and the detection method using polyacrylamide gel was labor intensive and tedious for routine DNA typing. Second, the reagent cost is high for the accurate determination of the polymorphic HLA-DRB1 gene contain-

Table 3. HLA-DRB1 allele frequencies in 112 unrelated Koreans using PCR-SSP

HLA DRB1 Allele	Observed Number	Allele Frequency
DRB1*0101	16	0.071
DRB1*0102	1	0.004
DRB1*0301	5	0.022
DRB1*0401	5	0.022
DRB1*0402	1	0.004
DRB1*0403	3	0.013
DRB1*0404	15	0.067
DRB1*0405	17	0.076
DRB1*0406	1	0.004
DRB1*0407	1	0.004
DRB1*0408	1	0.004
DRB1*0409	5	0.022
DRB1*0410	1	0.004
DRB1*0701	15	0.067
DRB1*0801	2	0.009
DRB1*0802	7	0.031
DRB1*0803	15	0.067
DRB1*0804	8	0.036
DRB1*0901	27	0.121
DRB1*1001	5	0.022
DRB1*1101	4	0.018
DRB1*1104	1	0.004
DRB1*1105	2	0.009
DRB1*1201	3	0.013
DRB1*1202	2	0.009
DRB1*1301	5	0.022
DRB1*1302	15	0.067
DRB1*1303	1	0.004
DRB1*1304	1	0.004
DRB1*1305	2	0.009
DRB1*1401	4	0.018
DRB1*1402	1	0.004
DRB1*1403	1	0.004
DRB1*1405	1	0.004
DRB1*1406	1	0.004
DRB1*1501	21	0.094
DRB1*1502	3	0.013
DRB1*1601	1	0.004
DRB1*1602	2	0.009
DRB1*1603	1	0.004
DRB1*1604	1	0.004

Table 4. Observed HLA-DRB1 Genotypes in 112 unrelated Koreans using PCR-SSP

HLA-DRB1 genotype	Number/ Frequency obs. obs.	HLA-DRB1 genotype	Number/ Frequency obs. obs.
DRB1*0101/0401	1/0.009	DRB1*0409	2/0.018
DRB1*0101/0409	1/0.009	DRB1*0410/1201	1/0.009
DRB1*0101/0701	4/0.036	DRB1*0701	1/0.009
DRB1*0101/0803	2/0.018	DRB1*0701/0804	1/0.009
DRB1*0101/0901	2/0.018	DRB1*0701/0901	3/0.027
DRB1*0101/1001	1/0.009	DRB1*0701/1201	1/0.009
DRB1*0101/1302	2/0.018	DRB1*0701/1302	1/0.009
DRB1*0101/1305	1/0.009	DRB1*0701/1501	1/0.009
DRB1*0101/1501	2/0.018	DRB1*0801/0901	1/0.009
DRB1*0102/0901	1/0.009	DRB1*0801/1101	1/0.009
DRB1*0301	1/0.009	DRB1*0802	1/0.009
DRB1*0301/0405	1/0.009	DRB1*0802/0901	1/0.009
DRB1*0301/1501	1/0.009	DRB1*0802/1101	1/0.009
DRB1*0301/1602	1/0.009	DRB1*0802/1302	1/0.009
DRB1*0401/0802	1/0.009	DRB1*0802/1604	1/0.009
DRB1*0401/0804	1/0.009	DRB1*0803	2/0.018
DRB1*0401/0901	1/0.009	DRB1*0803/0901	1/0.009
DRB1*0401/1501	1/0.009	DRB1*0803/1302	2/0.018
DRB1*0402/1502	1/0.009	DRB1*0803/1401	1/0.009
DRB1*0403	1/0.009	DRB1*0803/1501	2/0.018
DRB1*0403/0803	1/0.009	DRB1*0804/0901	1/0.009
DRB1*0404	2/0.018	DRB1*0804/1301	1/0.009
DRB1*0404/0701	1/0.009	DRB1*0804/1302	1/0.009
DRB1*0404/0803	1/0.009	DRB1*0804/1304	1/0.009
DRB1*0404/0804	1/0.009	DRB1*0901	2/0.018
DRB1*0404/0901	3/0.027	DRB1*0901/1302	1/0.009
DRB1*0404/1001	1/0.009	DRB1*0901/1501	5/0.045
DRB1*0404/1302	2/0.018	DRB1*1001	1/0.009
DRB1*0404/1401	2/0.018	DRB1*1101	1/0.009
DRB1*0405	3/0.027	DRB1*1104/1301	1/0.009
DRB1*0405/0701	1/0.009	DRB1*1105	1/0.009
DRB1*0405/0803	1/0.009	DRB1*1202/1501	1/0.009
DRB1*0405/0804	1/0.009	DRB1*1301	2/0.018
DRB1*0405/0901	2/0.018	DRB1*1302	1/0.009
DRB1*0405/1001	1/0.009	DRB1*1302/1401	1/0.009
DRB1*0405/1201	1/0.009	DRB1*1302/1501	2/0.018
DRB1*0405/1305	1/0.009	DRB1*1402/1403	1/0.009
DRB1*0405/1406	1/0.009	DRB1*1405/1601	1/0.009
DRB1*0405/1501	1/0.009	DRB1*1501	2/0.018
DRB1*0406/1202	1/0.009	DRB1*1501/1603	1/0.009
DRB1*0407/1602	1/0.009	DRB1*1502	1/0.009
DRB1*0408/0901	1/0.009		
Homozygosity (n=24) 0.214			
Heterozygosity (n=88) 0.786			

ing over 40 alleles. By using commercialized restriction enzymes, it is sometimes difficult to determine if

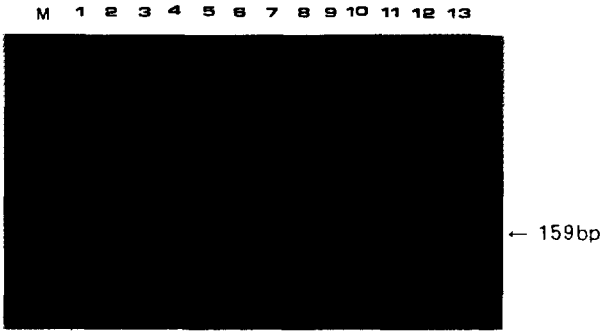

Fig. 1. Amplification product of DRB1*0901 control cell (Cell Number: 9075, Cell Name: DKB) by PCR-SSP.
M: Molecular Weight Marker (PhiX174 DNA/*Hinf*I); Lane 1: Primer mix 1; Lane 2: Primer mix 2; Lane 3: Primer mix 3; Lane 4: Primer mix 4; Lane 5: Primer mix 5; Lane 6: Primer mix 6; Lane 7: Primer mix 7; Lane 8: Primer mix 8; Lane 9: Primer mix 9; Lane 10: Primer mix 10; Lane 11: Primer mix 11; Lane 12: Primer mix 12 159 bp; Lane 13: Primer mix 13.

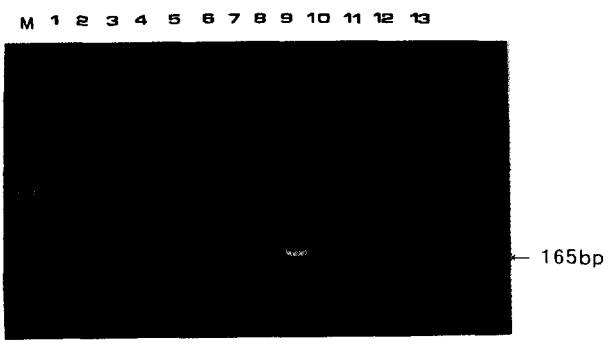

Fig. 2. Amplification product of DRB1*0803 control cell (Cell Number: 9070, Cell Name: LUY) by PCR-SSP.
M: Molecular Weight Marker (PhiX174 DNA/*Hinf*I); Lane 1: Primer mix 1; Lane 2: Primer mix 2; Lane 3: Primer mix 3; Lane 4: Primer mix 4; Lane 5: Primer mix 5; Lane 6: Primer mix 6; Lane 7: Primer mix 7; Lane 8: Primer mix 8; Lane 9: Primer mix 9 165 bps; Lane 10: Primer mix 10; Lane 11: Primer mix 11; Lane 12: Primer mix 12; Lane 13: Primer mix 13.

alleles have only 1~2 nucleic acid sequence variations, or if there are no restriction sites. Therefore, the PCR-RFLP method might be applicable in HLA-DQA1 and DQB1 genes which have only a small number of alleles, but not in HLA-DRB1 genes, which have a larger number of alleles. Moreover, in the PCR-SSO method, it is difficult to adjust the annealing temperature for hybridization and washing procedures. On the other hand, the PCR-SSP technique for HLA-DRB1 genotyping is more accurate, rapid, not labor intensive and more amenable to automation (Chia *et al.*, 1993, 1994) than other PCR-based methods, including PCR-RFLP and PCR-SSO (Hoshino, 1992; Olerup *et al.*, 1993; Bein *et al.*, 1994). This technique is also ideally suited for analyzing small numbers of samples simultaneously and can replace serological DR typing in routine clinical practice. Allelic variations within the

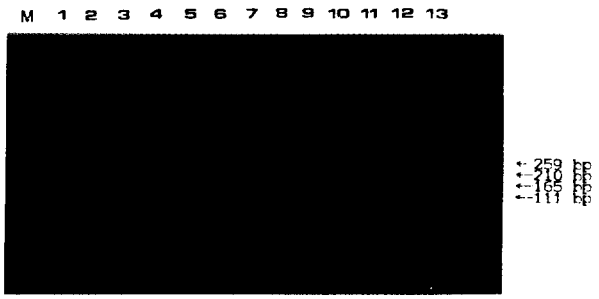

Fig. 3. Amplification products of DRB1*0803/*1302 by PCR-SSP.
M : Molecular Weight Marker (PhiX174 DNA/*Hinf*I); Lane 1 : Primer mix 1; Lane 2 : Primer mix 2; Lane 3 : Primer mix 3; Lane 4 : Primer mix 4; Lane 5 : Primer mix 5 111 bps; Lane 6 : Primer mix 6 259, 210 bps; Lane 7 : Primer mix 7; Lane 8 : Primer mix 8; Lane 9 : Primer mix 9 165 bps; Lane 10 : Primer mix 10; Lane 11 : Primer mix 11; Lane 12 : Primer mix 12; Lane 13 : Primer mix 13.

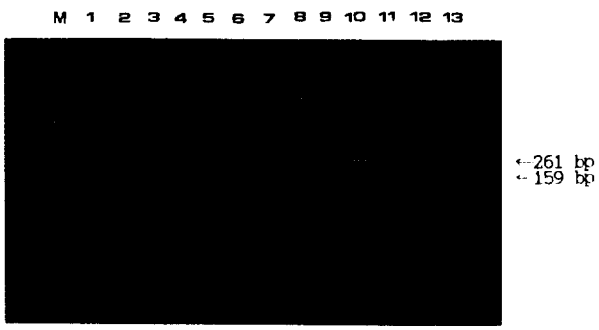

Fig. 4. Amplification products of DRB1*0802/*0901 by PCR-SSP.
M : Molecular Weight Marker (PhiX174 DNA/*Hinf*I); Lane 1 : Primer mix 1; Lane 2 : Primer mix 2; Lane 3 : Primer mix 3; Lane 4 : Primer mix 4; Lane 5 : Primer mix 5; Lane 6 : Primer mix 6; Lane 7 : Primer mix 7; Lane 8 : Primer mix 8; Lane 9 : Primer mix 9; Lane 10 : Primer mix 10 261 bps; Lane 11 : Primer mix 11; Lane 12 : Primer mix 12 159 bps; Lane 13 : Primer mix 13.

exon 2 region of the HLA-DRB1 gene carry distinct allospecificities and have been implicated in individual identification (Takahashi, 1989; Allen *et al.*, 1993), bone marrow transplantation (Tiercy and Friedel, 19 91), and specific HLA-associated diseases (Chia *et al.*, 1993), such as insulin-dependent diabetes mellitus (Catherine *et al.*, 1991), autoimmune diseases (Todd, 1988), Graves' disease (Grumet *et al.*, 1974; Cho *et al.*, 1987), rheumatoid arthritis (Ploski *et al.*, 1993) as well as transplantation immunity (Matsuno and Inoko, 1990; Beatty *et al.*, 1991). In conclusion, these results might be useful for database studies before application to individual identification and paternity determination among Koreans.

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