Journal of Biochemistry and Molecular Biology, Vol. 32, No. 6, November 1999, pp. 529–534

JOURNAL OF
Biochemistry
and
Molecular Biology

Asymmetric Polymerase Chain Reaction–Single-Strand Conformation Polymorphism (Asymmetric PCR–SSCP) as a Simple Method for Allele Typing of HLA-DRB

Joo Hyun Kang, Kyeong Hee Kim, Cheol-Young Maeng, and Kil Lyong Kim*

Peptide Engineering Research Unit, Protein Engineering Research Division,
Korea Research Institute of Bioscience and Biotechnology, P. O. Box 115, Yusong, Taejon 305-600, Korea

Received 21 June 1999, Accepted 16 July 1999

Asymmetric PCR and single-strand conformation polymorphism (SSCP) methods were combined to analyze human leukocyte antigen (HLA)-DRB allele polymorphism. Asymmetric PCR amplification was applied to generate single-stranded DNA (ssDNA) using the nonradioactive oligonucleotide primers designed for the polymorphic exon 2 region. The conformational differences of ssDNAs, depending on the allele type, were analyzed by nondenaturing polyacrylamide gel electrophoresis and visualized by ethidium bromide staining. The ssDNAs were clearly separated from double-stranded DNA without interference and obviously migrated depending on their allele type. This method was applied to the genomic DNA either from homozygous or from heterozygous cell lines containing the DR4 allele as template DNA using DR4-specific primers, and satisfying results were obtained. Compared to the standard PCR-SSCP method, this asymmetric PCR-SSCP method has advantages of increased speed, reproducibility, and convenience. Along with PCR-SSP or sequence-based typing, this method will be useful in routine typing of HLA-DRB allele.

Keywords: Asymmetric PCR, HLA class II DR molecule, Polymorphism, Single-Strand Conformation Polymorphism.

Introduction

The polymorphism encoded within the HLA class II region provides the structural basis for genetic control of the immune response (McDevitt and Chiritz, 1969). This region is divided into three subregions, DP, DQ, and DR, each encoding both α and β chains (Bell *et al.*, 1985). These chains form the heterodimeric cell surface glycoproteins that bind and present antigenic peptides to the $CD4^+$ T-cells to initiate immune responses. The HLA-DR region is further divided into the monomorphic α chain, and polymorphic β chains encoded by four different isotypic genes, DRB1, DRB3, DRB4, and DRB5 (Trowsdale *et al.*, 1991). The DRB1 genes show an extremely high polymorphism compared to other DRB genes. The polymorphism of DRB genes are mainly confined to the membrane-distal domain encoded by the second exon. The accurate identification of the HLA allele type is a prerequisite for organ or tissue transplantation and the determination of the functional roles of HLA molecules. The serological and cellular HLA typing methods have been replaced by the more accurate and simple genotyping methods using the polymerase chain reaction (PCR) (Thonnard *et al.*, 1995). Recently, PCR-sequence specific oligonucleotides (PCR-SSO) (Scharf *et al.*, 1991), nonradioactive reverse dot blot (Bugawan *et al.*, 1990), PCR-SSCP (Hoshino, 1992), PCR-restriction fragment length polymorphism (PCR-RFLP) (Nomura *et al.*, 1991; Lee, 1995), PCR-sequence specific primer (PCR-SSP) (Olerup *et al.*, 1993; Chia *et al*, 1994), and sequencing based typing (SBT) (Santamaria *et al.*, 1992) have been introduced as HLA genotyping methods.

SSCP analysis combined with PCR has become an important tool for DNA analysis, particularly in detecting point mutations (Orita *et al.*, 1990) and in DNA polymorphism typing (Carrington *et al.*, 1992; Hongyo *et al.*, 1993). SSCP analysis is based on the principle that single-stranded DNA (ssDNA) forms a secondary structure determined by intramolecular interactions (Orita *et al.*, 1989a). The ssDNA fragments containing a mutation, even a single-base substitution, have different secondary structures. These sequence-based secondary structures

* To whom correspondence should be addressed.
Tel: 82-42-860-4163; Fax: 82-42-860-4593
E-mail: kimkl@kribb4680.kribb.re.kr

affect the mobility of DNA during gel electrophoresis in nondenaturing conditions, and will show different mobilities in thermostatic conditions. The conventional PCR-SSCP method (Orita *et al.*, 1989b) uses radiolabeled PCR primers or nucleotides to generate radioactive double-stranded DNA (dsDNA) fragments. For analysis, the radioactive dsDNAs are denatured by heating and adding formamide, and then the resulting ssDNAs are resolved by electrophoresis in a sequencing gel. Several improved methods have been introduced to analyze ssDNA by using silver staining (Ainsworth *et al.*, 1991) or by using fluorescence-labeled primers (Makino *et al.*, 1992) instead of using radioactive reagents. However, these methods require expensive equipment or reagents. The asymmetric PCR method relies on using unequal amounts of primers (one is limited and the other is excess), or using only one primer to allow the selective accumulation of the sense or antisense ssDNA from the dsDNA templates depending on the primer used. The ssDNA fragments generated by asymmetric PCR are generally used for the direct applications as DNA sequencing templates (Ward *et al.*, 1993).

In this study, asymmetric PCR–SSCP analysis was applied to the detection of HLA-DRB gene polymorphism. The exon 2 or exon 3 region of HLA-DRB genes isolated from EBV-transformed human B-cell lines was analyzed using asymmetric PCR-SSCP with sequence specific primers. The results clearly demonstrated that asymmetric PCR-SSCP can be conveniently used for the analysis of highly polymorphic systems.

Methods

Total RNA and chromosomal DNA preparation Three EBV-transformed Korean B-cell lines, designated as CH, JS, and KT, were kindly provided by Dr. H. Han (The Catholic University of Korea). Each cell line has been serologically typed as DR4/11 for CH, DR4/14 for JS, and DR12/13 for KT. For the experimental control, two homozygous control B-cell lines, Wa (DRB1*0405) and Priess (DRB1*0401), were obtained from Dr. K. Sato (Asahikawa Medical College, Japan) and Dr. R. M. Chicz (Harvard University, USA), respectively. Cells were cultured in RPMI-1640 medium (Gibco-BRL, Grand Island, USA) supplemented with 10% FBS (Gibco-BRL), 2 mM glutamine, 100 U/ml of penicillin, and 100 mg/ml of streptomycin, and maintained in a humidified 5.5% CO_2 atmosphere at 37°C. Total cellular RNA was extracted from the 2×10^6 cultured B-cell lines using the guanidium isothiocyanate/ phenol extraction method (Chomczynsk and Sacchi, 1987). The chromosomal DNA was isolated from 1×10^6 cultured B-cell lines by proteinase K (Boehringer Mannheim, Mannheim, Germany) digestion, high salt treatment, and ethanol precipitation (Laird *et al.*, 1991). The purities and concentrations of nucleic acids were determined by measuring absorbances at 260 and 280 nm.

Reverse transcription–PCR (RT–PCR) and nucleotide sequence analysis The RT–PCR and cDNA subcloning were performed as described in a previous report (Maeng *et al.*, 1997) with minor modifications. Briefly, the oligonucleotide primers β1 and β2 were designed for the amplification of full-length DRB cDNA (Table 1). For the polymerase chain reaction, after initial denaturation at 95°C for 5 min, each cycle consisted of denaturation at 95°C for 1 min, annealing at 48°C for 1 min, and extension at 72°C for 1 min with a final extension at 72°C for 7 min. The amplified PCR products were subcloned into pGEM-T vector (Promega, Madison, USA). Nucleotide sequences were determined by fluorescence-labeled dideoxynucleotide termination reaction with an Applied Biosystems 373A automated DNA sequencer.

Enrichment of ssDNA by asymmetric PCR In order to set the asymmetric PCR condition and investigate ssDNA's electrophoretic patterns of several DRB alleles, the plasmids containing full-length DRB cDNA were used as templates (Fig. 1). Primers βc1 and βg2-1 were designed for universal DRB alleles and βg1 was specific for DR4 alleles (Table 1). PCRs were performed to amplify 192 bp fragments of the exon 2 region using βc1 and βg2-1 as primers, and the amounts of primers were 1 pmol of limiting primer and 20 pmol of excess primer in a total volume of 100 μl. After initial denaturation at 95°C for 5 min, each cycling condition for 45 cycles of amplification was at 95°C for 0.5 min, 52°C for 1.5 min, and 72°C for 1.5 min. Final extension was carried out at 72°C for 15 min. For ssDNA enrichment using genomic DNA, asymmetric PCR amplification was followed by symmetric PCR (Fig. 1). Symmetric PCRs were performed with 0.1 ng of genomic DNA and 20 pmol of each primer (βg1 and βg2-1) for 30 cycles (95°C for 0.5 min, 52°C for 1 min, and 72°C for 1.5 min) and the final extension reaction was at 72°C for 7 min. Using 100-fold diluted PCR products, we performed asymmetric PCRs with 20 pmol of excess primer and 1 pmol of limiting primer for 45 cycles (95°C for 0.5 min, 54°C

Table 1. Oligonucleotide primers used in this study.

Primer	Nucleotide Sequence	Uses	Remarks
β1	GTCCTCTTCTCCAGCATG	Cloning for full-length DRB cDNA	Sense
β2	GTCATCTGCACTTCAGCT	Cloning for full-length DRB cDNA	Antisense
βc1	ACGGAGCGGGTGCGGT	Universal for DRB allele	Sense
βg1	GGAGCAGGTTAAACATGAG	Specific for DR4 allele	Sense
βg2-1	CCCGTAGTTGTGTCTGCA	Universal for DRB allele	Antisense
p122	GGCAGCATTGAAGTCAGGTGGT	Amplification for exon 3 region of DR12 allele	Sense
pE3R	AACCTCTCCACTCCGAGGAACTGT	Amplification for exon 3 region of DR12 allele	Antisense

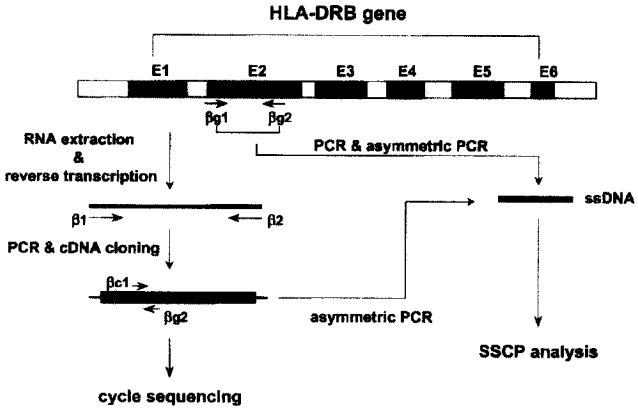

Fig. 1. Strategies of asymmetric PCR-SSCP for HLA-DRB polymorphism analysis.

for 1 min, and 72°C for 1.5 min) and the final extension reaction at 72°C for 15 min. In one experiment, asymmetric PCR was performed with DRB exon 3 specific primers p122 and pE3R. The reaction conditions were the same except that the annealing temperature was at 55°C.

SSCP analysis After asymmetric PCR amplification, the 10 μl PCR products were mixed with 2 μl loading buffer (10 mM Tris-Cl, 10 mM EDTA, pH 8.0, 0.25% bromophenol blue, 0.25% xylene cyanol, and 15% glycerol). The samples were loaded onto the 10% polyacrylamide-glycerol gel (49:1 acrylamide to bis-acrylamide cross-linking in 0.5× TBE containing 5% (v/v) glycerol; 18 × 16 × 0.075 cm) using a Pharmacia SE600 (Uppsala, Sweden) vertical slab gel apparatus. The electrophoresis reservoir was filled with 4 L of 0.5× TBE (45 mM Tris-borate, 1 mM EDTA, pH 8.0) and maintained at 10°C by connecting with a thermostatically controlled water bath. The gel was run at 10 mA constant current until the bromophenol blue reached the bottom. The running time was approximately 5 h. After electrophoresis, the gel was stained with a 0.5 μg/ml ethidium bromide (EtBr) solution and then destained with distilled water. The EtBr-stained bands were visualized using a 340 nm UV box and photographed.

Results and Discussion

The full-length DRB cDNAs of several alleles were isolated from four different B-cell lines by RT-PCR using β1 and β2 as sense and antisense primers, respectively. The nucleotide sequence of cDNA clones were then determined. DRB1*0406 and DRB1*11011 were obtained from CH, DRB1*1301, novel DR12 allele (DRB1*1206) (Maeng *et al.*, 1999), and DRB3*01012 from KT, and DRB1*0405 and DRB3*01012 from JS.

The asymmetric PCRs were performed using primers βc1 and βg2-1 to amplify the hypervariable region of exon 2 producing a 192 bp DNA fragment. The HLA-DRB clones were used as a template to produce SSCP markers. The optimal primer ratio for the asymmetric PCR was determined as 1 pmol of limiting primer and 20 pmol of excess primer by examining several reactions with various primer ratio combinations (unpublished data). Results of the SSCP analyses of asymmetric PCR products are shown in Fig. 2. The ssDNA fragment was resolved corresponding to its allele type and gave different mobility from dsDNA, which is also generated during asymmetric PCR. To compare the resolution of the ssDNA band with the conventional SSCP, we used loading buffer containing 95% formamide and 10 mM sodium hydroxide to denature the symmetrically amplified PCR products. However, we did not get any improved result and it might be due to the use of strong denaturants (data not shown). The SSCP pattern of DRB1*0405 from Wa (Fig. 2., lane 2 of A and B) and DRB3*01012 from JS (lane 5 of A and B) exactly matched that of DRB1*0405 from JS (lane 3 of A and B) and that of DRB3*01012 from KT (lane 6 of A and B). SSCP patterns of asymmetric PCR products of different alleles from the same DR subtype were also compared. The DR4 subtypes, DRB1*0401, DRB1*0405, and DRB1*0406, differ from each other by 3–5 nucleotides in the exon 2 region. For instance, DRB1*0405 differs from DRB1*0401 at codons 57 and 71, and from DRB1*0406 at codons 37, 57, and 74. These differences generate the remarkably distinctive SSCP patterns (Fig. 2, lanes 1, 2, and 4). Well-separated and reproducible SSCP patterns were also obtained using antisense oligonucleotide as an

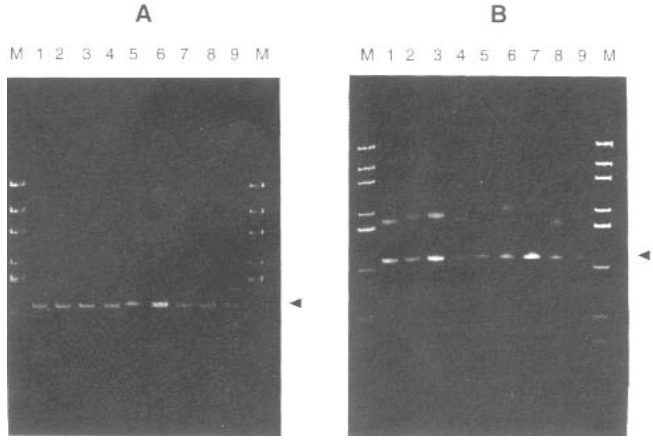

Fig. 2. SSCP analysis of various HLA-DRB alleles. The ssDNA of the 192 bp fragment was amplified by asymmetric PCR using plasmid containing DRB full-length cDNA with βc1 and βg2-1 as sense and antisense primers. The amplified samples were loaded onto the 10% polyacrylamide-5% glycerol gel (18 × 16 × 0.075 cm). After electrophoresis, the gel was stained with EtBr solution and then photographed. Panels A and B, either βc1 or βg2-1 was used as excess primer, respectively. Lane M, DNA marker (pUC19/*Hae*III digest); lane 1, DRB1*0401 from Priess; 2, DRB1*0405 from Wa; 3, DRB1*0405 from JS; 4, DRB1*0406 from CH; 5, DRB3*01012 from JS; 6, DRB3*01012 from KT; 7, DRB1*11011 from CH; 8, DRB1*1206 from KT; 9, DRB1*1301 from KT B-cell line. The arrowhead indicates the position of dsDNA.

excess primer under the same reaction conditions (Fig. 2, panel B). Therefore, we could select which strand gives the better separation, or analyze both strands independently for getting reliable results. The independently generated ssDNA fragments by asymmetric PCR gave an additional benefit in analyzing the SSCP pattern because of eliminating the possible interference between two ssDNAs generated from dsDNA by denaturation. We also applied the asymmetric PCR-SSCP to analyze cDNA transformants from heterozygous B-cell lines that might contain more than two different DRB alleles and could select clones which contain different DRB alleles without performing nucleotide sequencing for each cDNA clone (unpublished data).

The asymmetric PCR-SSCP was examined further with genomic DNA to confirm whether this method could be applied to HLA-DRB allele typing. In asymmetric PCR of heterozygotic genomic DNA, it is possible that DNA fragments are amplified from more than two different alleles by using universal primers βc1 and βg2-1. This may cause difficulties in SSCP analysis because of the appearance of multiple ssDNA bands. To solve this problem, we designed a DR4- subtype-specific oligonucleotide primer, βg1 (Table 1). The asymmetric PCR amplification of genomic DNA required two rounds of amplifications, symmteric and asymmetric, to obtain a sufficient amount of ssDNA fragment for EtBr-staining analysis. The dsDNA fragments from symmetric PCR were used for the asymmetric PCR template and the ssDNAs migrated depending on their allele type, like the above results (Fig. 3). In the SSCP analysis of genomic DNA, we used the asymmetric PCR products from cDNA clones (Fig. 2), as allele type markers. As we expected, the migration pattern of ssDNA from Wa genomic DNA (DRB1*0405) (Fig. 3, lane 2 of A and B) was identical to that of JS which also contains DRB1*0405 (Fig. 3, lane 3 of A and B), but was different from CH containing DRB1*0406 (Fig. 3, lane 6 of A and B). By matching these SSCP patterns with the markers, we could also confirm the results that the specific DRB allele type of the Wa and JS B-cell line was DRB1*0405 and that of CH was DRB1*0406. The novel allele of DR12 (DRB1*1206; Maeng *et al.*, 1999) was isolated from the KT cell line and its nucleotide sequences were different from DRB1*1201 at codons 149 and 161 in the exon 3 region of DR12. The polymorphism of DRB1*1206 compared to the DRB1*1201 allele in the exon 3 region was confirmed by the asymmetric PCR-SSCP technique using genomic DNA from the KT cell line as the template (Fig. 4).

The PCR-SSCP method is generally used in allele typing of MHC genes and in detecting mutations for disease diagnosis (Young and Darke, 1993; Bannai *et al.*, 1994; Pursall *et al.*, 1996; Majima *et al.*, 1997). Conventional PCR–SSCP methods require a denaturing step to generate ssDNA from symmetrically amplified

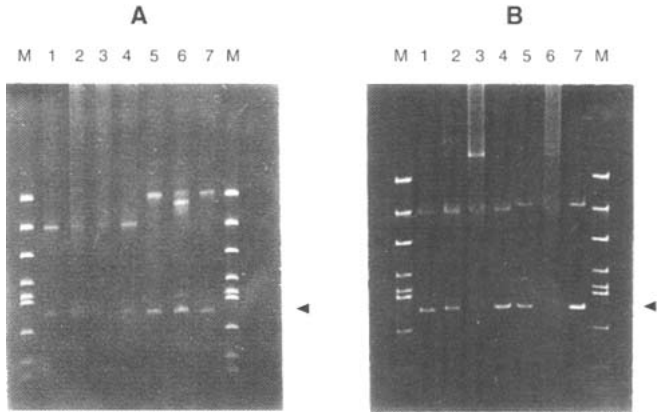

Fig. 3. Electrophoretic patterns of ssDNA of the 229 bp fragment generated with genomic DNA of B-cell lines. The ssDNA was amplified by asymmetric PCR followed by symmetric PCR using 0.1 ng of genomic DNA with βg1 and βg2-1 as sense and antisense primers. SSCP analysis conditions were as described in Fig. 2. Panels A and B, either βg1 or βg2-1 was used as excess primer, respectively. Lane M, DNA marker (pUC19/*Hae*III digest). Lanes 1 and 4 are ssDNA markers of DRB1*0405 from the cDNA clone; 5 and 7, ssDNA marker of DRB1*0406. Lanes 2, 3, and 6 were ssDNA amplified by genomic DNA of Wa, JS, and CH B-cell line, respectively. The position of the dsDNA is indicated with an arrowhead.

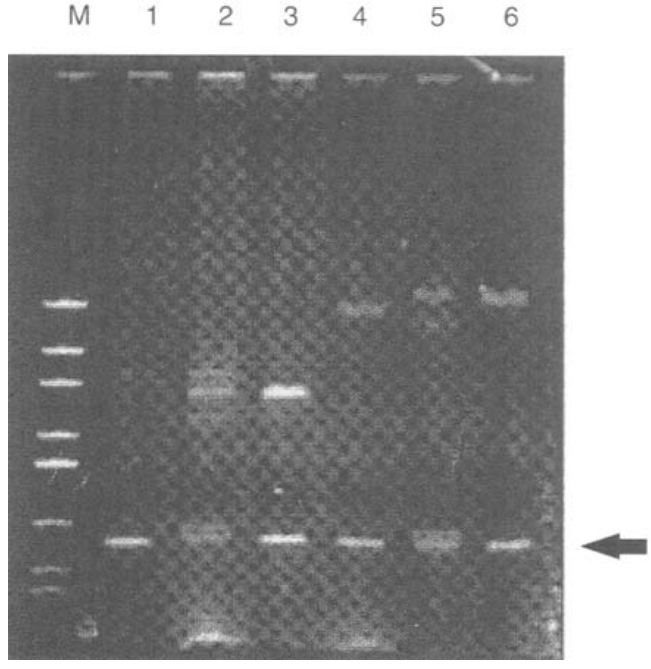

Fig. 4. Asymmetric PCR-SSCP analysis of HLA-DRB exon 3 region. Asymmetric PCR amplification and SSCP analysis were performed as described in Materials and Methods. Lane M, DNA marker (pUC19/*Hae*III digest). Lanes 1–3 and lanes 4–6 were amplified ssDNA using p122 or pE3R as an excess primer, respectively. Lanes 1, 4 and lanes 3, 6 were amplified ssDNA from plasmid containing full-length cDNA of DRB1*1201 (p1201) and DRB1*1206 (p1206), respectively. Lanes 2 and 5 were amplified ssDNA from KT genomic DNA. The arrow indicates the position of the dsDNA.

dsDNA products and use end-labeled primers either with γ-^{35}S-ATP or with fluorescent dye to amplify the signals (Hongyo *et al.*, 1993; Russell, 1994). However, these methods were somewhat complicated for routine HLA-DRB allele typing because of the low yield of ssDNA after denaturation and of using expensive or dangerous end-labeling materials. To overcome these problems, several SSCP methods have been reported (Saeki *et al.*, 1991; Hongyo *et al.*, 1993). These methods require the purchase of expensive additional equipment or use of silver staining techniques, and a denaturation step for ssDNA separation. Recently, the asymmetric PCR-SSCP method in polymorphism analysis of bovine MHC-DRB alleles has been reported (Russell, 1994). They analyzed the polymorphism of bovine MHC-DRB3 regions by an asymmetrically amplified PCR product using γ-^{35}S-ATP end-labeled primers. Instead of using an end-labeled primer with γ-^{35}S-ATP or fluorescent dye, which is expensive and produces biohazard wastes, we used nonradioactive oligonucleotide primers and convenient EtBr staining to visualize the DNA bands (Figs. 2, 3 and 4). Compared to the use of sequencing gels in SSCP analysis, we performed SSCP analysis with an easily manipulated gel format (0.75 mm thickness) or with a mini-gel format with the same resolution.

In this study, we developed an improved SSCP method which is simple, rapid, and used non-radioactive material, by combining with asymmetric PCR. The SSCP patterns with genomic DNA suggest that the asymmetric PCR-SSCP with group specific amplification is going to be useful in HLA allele discrimination. Generation of group-specific SSCP markers including DR4 would facilitate this method in routine work. Applying the asymmetric PCR-SSCP to blood samples and to generate markers will be performed in future work. In summary, we described the SSCP technique using asymmetric PCR as a practical tool for allelic polymorphism analysis and the determination of genotypes of HLA-DRB, even for the heterozygotes, and this method could be substituted for other typing methods in routine work.

Acknowledgments The authors are grateful to the Ministry of Science and Technology for their financial support through Research Grant HS1700.

References

Ainsworth, P. J., Surth, L. C. and Couler-Mackie, M. B. (1991) Diagnostic single strand conformation polymorphism (SSCP): A simplified non-isotopic method as applied to a Tay-Sachs B1 variant. *Nucleic Acid Res.* **19**, 405–406.

Bannai, M., Tokunaga, K., Lin, L., Kuwata, S., Mazda, T., Amaki, I., Fujisawa, K. and Juji, T. (1994) Discrimination of human HLA-DRB1 alleles by PCR–SSCP method. *Eur. J. Immunogenet.* **21**, 1–9.

Bell, J. I., Denney, D. W. and McDevitt, H. O. (1985) Structure and polymorphism of murine and human class II major histocompatibility antigens. *Immunol. Rev.* **84**, 51–71.

Bugawan, T. L., Begovich, A. B. and Erlich, H. A. (1990) Rapid HLA-DPB typing using enzymatically amplified DNA and nonradioactive sequence-specific oligonucleotide probes. *Immunogenetics* **32**, 231–241.

Carrington, M., Miller, T., White, M., Gerrard, B., Stewart, C., Dean, M. and Mann, D. (1992) Typing of HLA-DQ A1 and DQ B1 using DNA single strand conformation polymorphism. *Hum. Immunol.* **33**, 208–212.

Chia, D., Terasaki, P. I., Chan, H., Acalinovich, A., Maruya, E., Saji, H. and Ware, K. (1994) A new simplified method of gene typing. *Tissue Antigens* **44**, 300–305.

Chomczynski, P. and Sacchi, N. (1987) Single step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**, 156–159.

Hongyo, T., Buzard, G. S., Calvert, R. J. and Weghorst, C. M. (1993) 'Cold SSCP': a simple, rapid and non-radioactive method for optimized single-strand conformation polymorphism analyses. *Nucleic Acids Res.* **21**, 3637–3642.

Hoshino, S. (1992) Polymerase chain reaction–single-strand conformation polymorphism analysis of polymorphism in DPA1 and DPB1 genes: a simple, economical, and rapid method for histocompatibility testing. *Hum. Immunol.* **33**, 98–107.

Laird, P. W., Zijderveld, A., Linders, K., Rudnicki, M. A., Jaenisch, R. and Berns, A. (1991) Simplified mammalian DNA isolation procedure. *Nucleic Acids Res.* **19**, 4293.

Lee, K. O (1995) The genotyping of HLA-DQA1 and HLA-DQB1 using polymerase chain reaction-restriction fragment length polymorphism combined with group specific primers among Koreans. *Mol. Cells* **5**, 35–40.

Maeng, C.-Y., Kang, J. H., Park, J.-H., Hahm, K.-S. and Kim, K. L. (1997) Cloning and nucleotide sequence analysis of HLA-DRA*0101 and DRB1*0405 alleles. *Kor. J. Immunol.* **19**, 17–27.

Maeng, C.-Y., Kim, K. H., Kang, J. H., Han, H. and Kim K. L. (1999) A novel HLA-DR12 allele (DRB1*1206) found in a Korean B-cell line. *Tissue Antigens* **53**, 516–518.

Majima, T., Tsujiuchi, T., Tsutsumi, M., Tsunoda, T. and Konishi, Y. (1997) Mutations of K-ras but not p53 genes in biliary duct and pancreatic duct carcinomas induced in hamsters by cholecystoduodenostomy with dissection of the common duct followed by N-nitrosobis(2-oxopropyl)amine. *Cancer Lett.* **118**, 47–53.

Makino, R., Yazyu, H., Kishimoto, Y., Sekiya, T. and Hayashi, K. (1992) F-SSCP: Fluorescence-based polymerase chain reaction–single strand conformation polymorphism (PCR–SSCP) analysis. *PCR Methods Appl.*, **2**, 10–13.

McDevitt, H. O. and Chiritz, A. (1969) Genetic control of the antibody response; relationships between immune response and histocompatibility (H-2) type. *Science* **163**, 1207–1208.

Nomura, N., Ota, M., Tsuji, K. and Inoko, H. (1991) HLA-DQB1 genotyping by a modified PCR–RFLP method combined with group-specific primers. *Tissue Antigens* **38**, 53–59.

Olerup, O., Aldener, A. and Fogdell, A. (1993) HLA-DQB1 and -DQA1 typing by PCR amplification with sequence-specific primers (PCR–SSP) in 2 hours. *Tissue Antigens* **41**, 119–134.

Orita, M., Iwahara, H., Kanazawa, H., Hayashi, K. and Sekiya, T. (1989a) Detection of polymorphisms of human DNA by gel electrophoresis as single strand conformation polymorphism. *Proc. Natl. Acad. Sci. USA* **86**, 2766–2779.

Orita, M., Suzuki, Y., Sekiya, T. and Hayashi, K. (1989b) Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics* **5**, 874–879.

Orita, M., Sekiya, T. and Hayashi, K. (1990) DNA sequence polymorphisms in Alu repeats. *Genomics* **8**, 271–278.

Pursall, M. C., Clay, T. M. and Bidwell, J. L. (1996) Combined PCR-heteroduplex and PCR–SSCP analysis for matching of HLA-A, -B, and -C allotypes in marrow transplantation. *Eur. J. Immunogenet* **23**, 41–53.

Russell, G. C. (1994) Improved single-strand conformation polymorphism analysis by asymmetric polymerase chain reaction with end-labeled primers. *Genet. Anal. Tech. Appl.* **11**, 24–27.

Saeki, Y., Ueno, S., Yorifuji, S., Sugiyama, Y., Ide, Y. and Matsuzawa, Y. (1991) New mutant gene (transthyretin Arg58) in cases with hereditary polyneuropathy detected by non-isotope method of single strand conformation polymorphism analysis. *Biochem. Biophys. Res. Commun.* **180**, 380–385.

Santamaria, P., Boycejacino, M. T., Lindstrom, A. L., Barbosa, J. J., Faras, A. F. and Rich, S. S. (1992) HLA class II "typing": direct sequencing of DRB, DQB, and DQA genes. *Hum. Immunol.* **33**, 69–81.

Scharf, S. J., Griffith, R. L. and Erlich, H. A. (1991) Rapid typing of DNA sequence polymorphism at the HLA-DRB1 locus using the polymerase chain reaction and nonradioactive oligonucleotide probes. *Hum. Immunol.* **30**, 190–201.

Thonnard, J., Deldime, F., Heusterspreute, M., Delepelt, B., Hanon, F., Bruyere, M. and Phillippe, M. (1995) HLA class II genotyping: two assay systems compared. *Clin. Chem.* **41**, 553–556.

Trowsdale, J., Ragoussis, J. and Campbell, R. D. (1991) Map of the human MHC. *Immunol. Today* **12**, 443–446.

Ward, R. L., Hawkins, N. J., Wakefield, D., Atkinson, K. and Biggs, J. C. (1993) Production of a functional single-chain Fv fragment from the pan-leukocyte antibody WM65 using splicing by asymmetric PCR. *Exp. Hematol.* **21**, 660–664.

Young, N. T. and Darke, C. (1993) Allelic typing of the HLA-DR4 group by polymerase chain reaction-single strand conformation polymorphism analysis. *Hum. Immunol.* **37**, 69–74.