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Brief Communication

A simple ABO genotyping by PCR using sequence-specific primers with mismatched nucleotides

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ABSTRACT

In forensics, the specific ABO blood group is often determined by analyzing the ABO gene. Among various methods used, PCR employing sequence-specific primers (PCR-SSP) is simpler than other methods for ABO typing. When performing the PCR-SSP, the pseudo-positive signals often lead to errors in ABO typing. We introduced mismatched nucleotides at the second and the third positions from the 3′-end of the primers for the PCR-SSP method and examined whether reliable typing could be achieved by suppressing pseudo-positive signals. Genomic DNA was extracted from nail clippings of 27 volunteers, and the ABO gene was examined with PCR-SSP employing primers with and without mismatched nucleotides. The ABO blood group of the nail clippings was also analyzed serologically, and these results were compared with those obtained using PCR-SSP. When mismatched primers were employed for amplification, the results of the ABO typing matched with those obtained by the serological method. When primers without mismatched nucleotides were used for PCR-SSP, pseudo-positive signals were observed. Thus our method may be used for achieving more reliable ABO typing.

1. Introduction

The ABO gene is located at the 9th chromosome and is composed of seven exons that encode a transferase that catalyzes the transfer of sugars to glycosphingolipids or glycoproteins, resulting in the formation of the ABO hapten [1,2]. Numerous single nucleotide polymorphisms (SNPs) have been found throughout the ABO gene. A few SNPs identified within exon 6 and exon 7 give rise to a protein with an altered amino acid sequence leading to a change in the substrate specificity of the enzyme. A transferase with altered specificity synthesizes a different hapten. Therefore, in order to identify the ABO blood group, SNPs need to be analyzed.

Various methods have been reported that discriminate SNPs in the ABO gene and they can be used for ABO typing. These methods include PCR-restriction fragment length polymorphism (PCR-RFLP) [3–7], PCR employing sequence-specific primers (PCR-SSP) [8–14], single-strand conformation polymorphism (PCR-SSCP) [15–17], and DNA chip [18]. Among these, PCR-SSP has been extensively used owing to the simplicity of its methodology, which requires only the use of general-purpose equipment. When ABO blood typing is performed using PCR-SSP, pseudo-positive signal originating from unexpected primer extension reactions caused by noncomplementary annealing of a primer to the SNP site must be minimized to obtain reliable results. Several studies have attempted to reduce the pseudo-positive signals in PCR-SSP primarily by incorporating mismatched nucleotides into the primers [10–13]. Other strategies have included the addition of various chemicals to the PCR mixture [8,9,13]. However, these methods needed optimization and general guidelines have not been established. In a recent study, allele-specific primers with mismatched nucleotides near the 3′ end were employed in an attempt to determine the best position and the optimal number of mismatches to be used in PCR-SSP [19]. The authors found that the pseudo-positive signals were low when the primer containing a 3′-terminal nucleotide that recognized the SNP and the next two nucleotides formed mismatch pairings with the template nucleotides.

In this study, we attempted to establish a simple and robust PCR-SSP-based ABO genotyping method. We first examined the compatibility of various primers, designed as described above, for the ABO genotyping. Because introduction of mismatched nucleotides to the primers of the PCR-SSP could not prevent the pseudo-positive signals completely [19], we used two-step PCR and precisely controlled the amount of template DNA for the second PCR step. We used real-time PCR to compare the amplification efficiencies between reactions that used mismatched primers and those employing primers without mismatched nucleotides. Secondly, we performed the ABO typing with amplified PCR-SSP

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Table 1
Sequences of common primers for the first and the second PCR amplification of ABO gene.

Primer	Sequence (5′–3′)
Exon6-f	CAGAAGCTGAGTGGAGTTTCCAGG
Exon6-r	CTGAACTGCTCGTTGAGGATGTCG
Exon7-f	AAGGACGAGGGCGATTTCTACTAC
Exon7-r	CGTTGGCCTGGTCGACCATCATG

products using agarose gel electrophoresis. We used the primer sets that amplified SNP261 and SNP796 regions to distinguish A, B, and O alleles [20].

Table 2
Sequences of forward primers for the PCR-SSP.

Primers	Sequence (5′-3′)
261-AB	AGTAGGAAGGATGTCCTCGTGGT**G**
261-O	GCAGTAGGAAGGATGTCCTCGTGGT**A**
261-AB-m	GTGCAGTAGGAAGGATGTCCTCGTG<u>AC</u>**G**
261-O-m	GTGCAGTAGGAAGGATGTCCTCGTG<u>AC</u>**A**
796-AO	AGGACGAGGGCGATTTCTACTAC**C**
796-B	AGGACGAGGGCGATTTCTACTAC**A**
796-AO-m	AAGGACGAGGGCGATTTCTACT<u>CT</u>**C**
796-B-m	AAGGACGAGGGCGATTTCTACT<u>CT</u>**A**

Bold nucleotide is complementary to the SNP nucleotide and underlined nucleotide is noncomplementary to the template nucleotide.

2. Materials and methods

2.1. Samples and DNA extraction

This study was approved by the institutional ethics committee. Nail clippings from 27 Japanese volunteers were collected with informed consent. The ABO phenotypes of the samples were determined using an adsorption elution method. DNA was extracted from the samples using a QIAamp DNA Investigator Kit (QIAGNE, Hilden, Germany) and quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Wilmington, DE).

2.2. The first amplification of the ABO gene with two-step PCR

A 195-bp (A, B allele) or a 194-bp (O allele) region containing exon 6 of the ABO gene harboring SNP 261 and a 112-bp region from exon 7 harboring SNP 796 were amplified separately with PCR. Typically, a total volume (25 μL) of the PCR mixture contained 0.625 units of TaKaRa Taq Hot start DNA polymerase (Takara Biomedical, Otsu, Japan), 2.5 μL of 10× PCR buffer, 0.2 mM deoxynucleotide triphosphates (dNTPs), 0.5 μL each of forward and reverse primers (each 100 μM), and 12.5 ng genomic DNA. Following preheating at 95 °C for 10 min, 50 cycles of amplification at 95 °C for 10 s, 58 °C for 10 s, and 72 °C for 10 s were carried out. This was followed by a post-incubation at 72 °C for 5 min to end the reaction. PCR was carried out using a GeneAmp PCR System 9600 (Applied Biosystems, Foster City, CA). Sequences of the primers used are shown in Table 1. After confirmation of the PCR products by agarose gel electrophoresis on a 3% Metaphor Agarose (Bio Whittaker Molecular, Rockland, ME) gel, the PCR products were purified using a QIAquick PCR Purification kit (QIAGEN) and

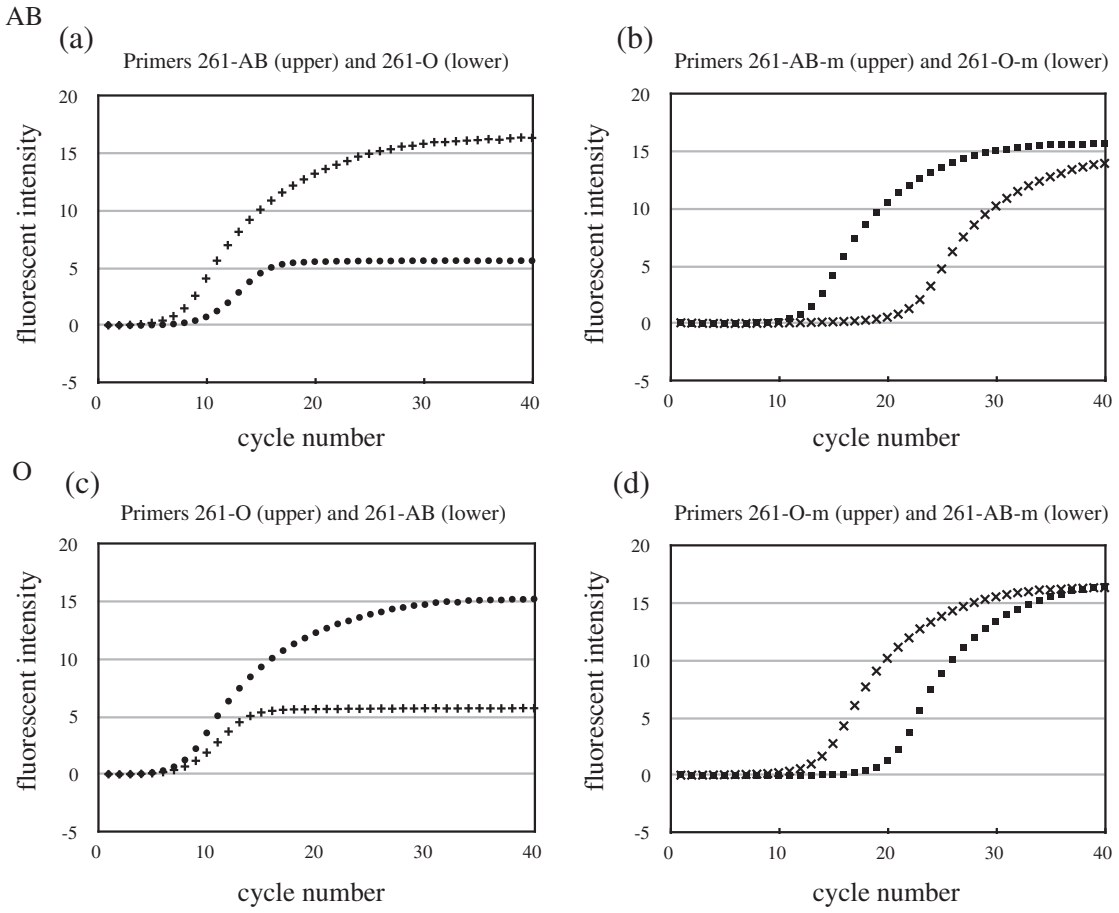

Fig. 1. Amplification efficiencies of the PCR-SSP at the SNP261. One sample derived from the AB blood type (a) and (b) and the other sample derived from the O blood type (c) and (d) were amplified with PCR-SSP. Forward primers used were +: 261-AB, ●: 261-O, ■: 261-AB-m, ×: 261-O-m.