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Forensic ABO blood grouping by 4 SNPs analyses using an ABI PRISM® 3100 genetic analyzer

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Abstract. This paper reports effective PCR-based methods, such as sequence-specific primers with a positive control (PCR-SSPPC) and confronting two pairs of primers (PCR-CTPP) for forensic ABO groupings using fragment analysis by ABI PRISM® 3100 genetic analyzer. The method allows the well-established base changes at four nucleotide positions 261, 796, 802, and 803 to be assayed, so that reliable group prediction is established by the presence of three representative alleles such as A, B, and O. As a result, all common alleles in the database were correctly determined by our methods.

Keywords: ABO blood grouping; PCR-SSPPC; PCR-CTPP; Fragment analysis; Phenotype

1. Introduction

Since 1900, ABO blood grouping has been determined by serological technique. In 1990, the molecular basis of glycosyl transferase genes involved in the ABO blood group system was determined [1], and 114 alleles based on the nucleotide sequencing have been identified and described in the blood group antigen gene mutation database [2]. There are some differences between the serological ABO typing and ABO typing using DNA analyses, and we now face the problem of whether to examine all of these alleles as part of the forensic ABO blood grouping strategies. However, it is exceedingly important to classify four phenotypes such as A, B, O and AB to establish forensic ABO grouping by the analysis of nucleotide sequencing. We previously proposed the analysis of four SNPs at nucleotide positions 261, 796, 802 and 803 to reflect serologic specificity [3,4]. This paper reports effective PCR-based methods, such as sequence-specific primers with a

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Table 1
Primer pairs for each PCR (Exon6 and Exon7)

	Primer	Sequence	FC*
Exon6 (261del)	K1	tail-CAGCTCCATGTGACCGCACGC	2 pmol
	NED-K2**	NED-TCGCCACTGCCTGGGTCTCTACC	2 pmol
	K31	tail-AGTAGGAAGGATGTCCTCGTGGAAC	20 pmol
Exon7 (796A, 802G, 802C)	S1	tail-AGCCGGGAGGCCTTCACCTA	2 pmol
	6FAM-S5**	6FAM-TGAGCCGCTGCACCTCTTGCA	2 pmol
	S20	tail-GACGAGGGCGATTTCTACTAGAT	10 pmol
	S3	tail-CGATTTCTACTACCTGGGCAG	10 pmol
	NED-S7**	NED-CCGACCCCCCGAAGAACGC	10 pmol
Exon7 (803G)	S1	Same as described above	2 pmol
	6FAM-S5**	Same as described above	2 pmol
	6FAM-S60**	6FAM-ACCGACCCCCCGAAGAATCC	10 pmol
Exon7 (803C)	S1	Same as described above	2 pmol
	6FAM-S5**	Same as described above	2 pmol
	VIC-S9**	VIC-ACCGACCCCCCGAAGAACG	10 pmol

* Final concentration for PCR.

** Dye-labeled primer.

positive control (PCR-SSPPC) and confronting two pairs of primers (PCR-CTPP) for forensic ABO groupings using fragment analysis by ABI PRISM® 3100 genetic analyser (Applied Biosystems).

2. Materials and methods

DNA samples were extracted by QIAamp® DNA Mini Kit (QIAGEN) from blood. Four kinds of PCR, consisting of examinations of Exon6 (261del), Exon7 (796A, 802G, 802C), Exon7 (803G), and Exon7 (803C), were performed. Amplification was accomplished in 25 μL of reaction mixture, which contained 10 ng extracted DNA, 1× PCR buffer (Invitrogen), 1.5 mM $MgCl_2$, 0.2 mM of dNTPs, and 2.5 U of Platinum Taq polymerase (Invitrogen). Pairs of primers used for PCR are shown in Table 1. Amplification was carried out according to the following reaction cycles: Initial denaturation step was performed for 11 min at 95 °C, followed by 30 cycles consisting

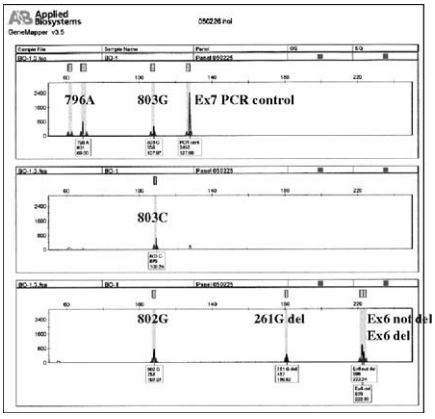

Fig. 1. Fragment chart of PCR products obtained from B phenotype. Eight fragment peaks (796A, 803G, Ex7 PCR control, 803C, 802G, 261G del, Ex6 not del, and 261 del) were observed in both B and O alleles.

Table 2
Typing patterns of ABO fragments

Alleles for ABO grouping	Alleles from data base (Ref. [2])	261	796	802	803
Common type					
A allele	A101-106, A201-206, A301-303, Ax01-08, Aw01-07.Aw09-10, Ael01-04, Am	G	C	G	G
B allele	B101-107, B301-304, B(A)01-03, Bx01, Bel01-03, Bw01-10	G	A	G	C
O allele	O01-02, O04-07, O09-13, 017-O18, O21-23, O25-O36, O43-47	del	C	G	G
Rare type					
identical to A allele	OO8, O14, O15, O19, O20, O39, O40	G	C	G	G
A or O allele	AW08, O03(O2)	G	C	A	G
AB allele	cisAB-1,cisAB-2	G	C	G	C
identical to B allele	cisAB-3	G	A	G	C
O allele	O24, O41, O42	del	A	G	C

of denaturation for 1 min at 94 °C, annealing for 1 min at 60 °C (for Exon6 at 63 °C instead of at 60 °C), extension for 1 min at 72 °C. Then, the final extension step was performed for 60 min at 60 °C. PCR products were analyzed by ABI Prism® 3100 Genetic Analyzer and Gene Mapper Software (Applied Biosystems).

3. Results and discussion

Fig. 1 shows an example of fragment chart patterns obtained from a DNA sample belonging to B phenotype. ABO grouping was performed based on differences among four SNPs shown in Table 2.

Our data showed that 105 of 114 alleles in the database [2] corresponded to the three major alleles by assaying the four nucleotide positions 261, 796, 802, and 803. The remaining 9 alleles, the two (Aw08 and O03, initially called O^2), and the seven (O08, initially called O^3, O14, initially called O301, O15, initially called O302, O19, initially called R102, O20, initially called R103, O39 and O40) are difficult to distinguish as to involvement in an A or O allele. These O alleles differ from the common O allele by the absence of one nucleotide deletion of a G at position 261. Although these frequencies are extremely low in Japan, more detailed investigation accompanied by serological prevalence data will be necessary. The common O alleles share a deletion of 261G. This deletion induces a frameshift and creates a premature stop codon. The O allele corresponds to a silent allele of the ABO gene. Determining the silent allele without deletion only by analysis of the nucleotide sequence is difficult. Accordingly, both serologic and PCR-based testing should be applied to classify the four phenotypes, such as A, B, AB and O, in the practice of forensic ABO blood grouping. In addition, both PCR-SSPPC and PCR-CTPP methods using fragment analysis by ABI PRISM® 3100 genetic analyzer are an effective method, because these methods include a PCR control to examine whether the target DNA obtained from the forensic specimen can be amplified or not.

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