International Congress Series 1261 (2004) 556–558

www.ics-elsevier.com

AB0 blood group genotyping of multiple single nucleotide polymorphisms using SNaPshot

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Abstract. A novel reproducible strategy to type the AB0 blood group system by the minisequencing of five nucleotide positions using SNaPshot (Applied Biosystem, USA) is described. This new technique has been successfully applied on 30 blood donors and the results agree with the known serologically based predicted genotypes.

Keywords: AB0; Polymorphism; PCR; Single nucleotide polymorphism (SNP); Minisequencing

1. Introduction

AB0 gene on chromosome 9 consists of seven exons ranging in size from 28 to 688 bp and mutations critical for AB0 blood group phenotypes have predominantly been found in exons 6 and 7 [1]. We describe here a novel reproducible strategy based on a duplex PCR assay followed by multiplex PCR minisequencing protocol that simultaneously allows genotyping of the well-established single nucleotide polymorphisms (SNPs) at five nucleotide positions 261, 297, 467, 646 and 703 to reveal six common alleles (A1, A2, B, 01, 01V and 02). We tested this technique on 30 blood donors with known serologically based predicted genotypes in family studies.

2. Materials and methods

Blood samples were collected from 30 healthy individuals with known serologically AB0 genotypes in family studies. Genomic DNA was isolated from 200 μl of whole blood using QIAamp DNA Mini Kit (Qiagen,Germany). All primer sequences and final concentrations are listed in Table 1. PCR amplification of the 10 ng of DNA template was performed in a 25-μl reaction mixture with 1.5 mM $MgCl_2$, 200 μM dNTPs, 1 × Gold Buffer (Applied Biosystem, USA) and 1 U of Taq Gold Polymerase (Applied Biosystem) at 10 min at 95 °C for one cycle; 1 min at 94 °C, 1 min at 59 °C, 1 min at 72 °C for 34 cycles

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0531-5131/
doi:10.1016/j.ics.2003.11.012

Table 1
Primer sequences and concentrations used

Locus	Sequence	Final concentration (μM)
AB0 6F 20 mer	5′ -GCCTCTCTCCATGTGCAGTA-3′	0.3
AB0 6R 20 mer	5′ -AACCCAATGGTGGTGTTCTG-3′	0.3
AB0 7F 20 mer	5′ -TGGCTTTCCTGAAGCTGTTC-3′	0.3
AB0 7R 19 mer	5′ -GATGTAGGCCTGGGACTGG-3′	0.3
Extension primers		
261 27 mer F	5′ -$(T)_8$GGAAGGATGTCCTCGTGGT-3′	0.15
297 21 mer R	5′ -GTTGAGGATGTCGATGTTGAA-3′	0.4
467 38 mer F	5′ -$(T)_{16}$TACTATGTCTTCACCGACCAGC-3′	0.5
646 44 mer F	5′ -$(T)_{24}$GCGTGGACGTGGACATGGAG-3′	0.4
703 33 mer F	5′ -$(T)_{17}$CGGCACCCTGCACCCC-3′	0.075

and 7 min of final extension at 72 °C Minisequencing by SNaPshot Multiplex kit (Applied Biosystem) and post-extension treatment were performed as described by manufacturer. All samples were run on ABI PRISM 310 Genetic Analyzer (Applied Biosystem).

3. Results

The DNA template of 131/132 bp on exon 6 and 389 bp on exon 7 was successfully amplified in a duplex PCR reaction. Each extension primer was first tested by uniplex reaction then multiplex SNaPshot was utilized and the interpretation of the electrophoretic pattern from minisequencing (Fig. 1) with a table of reference (Table 2) allowed to assign

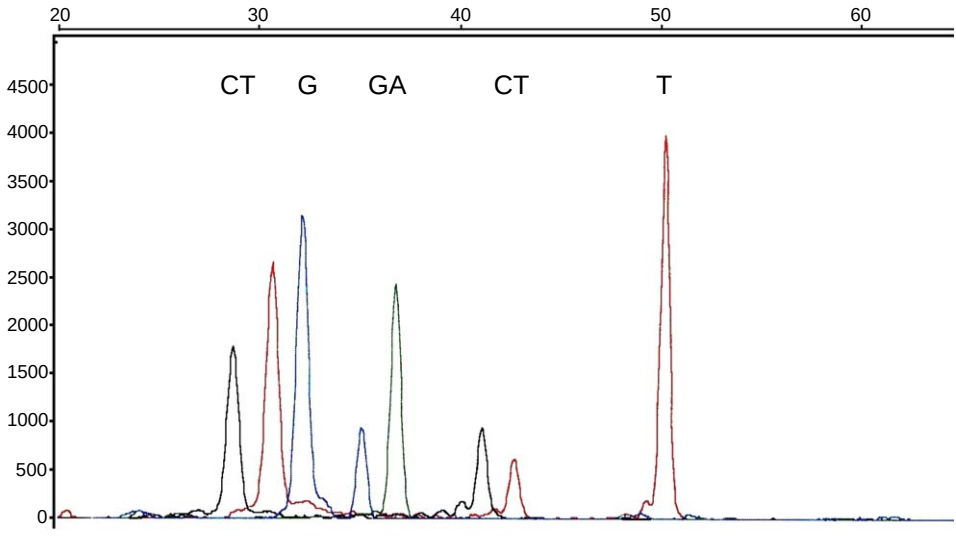

Fig. 1. AB0 blood group genotyping of multiple single nucleotide polymorphisms using SNaPshot: A2-B genotype (CT G GA CT T).

Table 2
AB0 genotype interpretation by minisequencing

Genotype	R 297 (22 bp)	F 261 (28 bp)	F 703 (34 bp)	F 467 (39 bp)	F 646 (45 bp)
A1 A1	T	G	G	C	T
A2 A2	T	G	G	T	T
B B	C	G	A	C	T
01 01	T	A	G	C	T
01V 01V	C	A	G	C	A
02 02	C	G	G	C	T
A1 A2	T	G	G	C T	T
A1 B	C T	G	G A	C	T
A1 01	T	G A	G	C	T
A1 01V	C T	G A	G	C	T A
A1 02	C T	G	G	C	T
A2 B	C T	G	G A	C T	T
A2 01	T	G A	G	C T	T
A2 01V	C T	G A	G	C T	T A
A2 02	C T	G	G	C T	T
B 01	C T	G A	G A	C	T
B 01V	C	G A	G A	C	T A
B 02	C	G	G A	C	T
01 01V	C T	A	G	C	T A
01 02	C T	G A	G	C	T
01V 02	C	G A	G	C	T A

the AB0 genotype without difficulties on 30 blood donors with known serologically based predicted genotypes in family studies.

4. Discussion

Via SNaPshot multiplex reaction we have developed a reproducible strategy to type the AB0 blood group system based on a duplex PCR assay followed by multiplex primers extension analysis. The well-established SNPs investigation at five nucleotide positions 261, 297, 467,646 and 703 permits the identification of A1, A2, B, 01, 01V and 02 alleles.

This method shows several advantages: first, one reaction mixture is required for DNA template amplification and one for minisequencing analysis that is a viable system for typing candidate SNPs at a moderate throughput level. Second, it is more practical, rapid than RFLP analysis, resulting unambiguous the interpretation of AB0 genotypes with a table of reference.

The molecular approach for typing AB0 blood group allows the direct determination of the AB0 genotype without family studies on the same DNA sample that can be also typeable for STRs and it may provide an additional information in identification case-works where AB0 blood group record is the only available data.

The present method may be very useful for personal identification as well as for paternity testing in forensic science.

Reference

[1] M.L. Olsson, M.A. Chester, Polymorphism and recombination events at the ABO locus: a major challenge for genomic AB0 blood grouping strategies, Transfus. Med. 11 (2001) 295–313.