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Forensic Science International: Genetics Supplement Series 1 (2008) 66–67
FSI GENETICS SUPPLEMENT SERIES
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Research article

Simultaneous detection of ABO and Secretor-nonsecretor blood groups from forensic biological samples by fragment analysis

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Received 15 August 2007; accepted 7 October 2007

Abstract

This paper describes the simultaneous detection of ABO and Secretor-nonsecretor (SE) blood groups from forensic biological samples by fragment analysis using the ABI PRISM® 3130 genetic analyzer. The method allows the assay of well-known base changes at three nucleotide positions 261, 796 and 803 on cDNA of the ABO gene, and at 385 and 428 on cDNA of SE gene and a SE pseudo gene, so that reliable group prediction is established by the presence of representative alleles. As a result, simultaneous detection of ABO and SE blood groupings from biological samples was correctly determined by our methods.
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Keywords: ABO blood group; Secretor-nonsecretor blood group; Genotyping; Fragment analysis

1. Introduction

ABO and Secretor-nonsecretor (SE) blood groups are very useful information concerning forensic samples such as body fluid spots; however, it is occasionally difficult to decide ABO grouping by serological methods because of the small quantity or nonsecretor typing.

In our previous paper, we have developed forensic ABO blood grouping by 4 SNPs analyses using genetic analyzer [1]. This is an effective method using DNA for inferring the four common ABO phenotypes from ABO and its subtype bloodstains [2]. We also developed a multiplex PCR with confronting two pair primers for SE genotyping classified into *Se*, *se*1, *se*2, and *se*5 alleles [3].

The purpose of this study is to establish the simultaneous detection of ABO and SE blood groupings using DNA materials from forensic biological samples.

2. Materials and methods

DNA samples were extracted by QIAamp® DNA Mini Kit (QIAGEN) from blood, saliva and urine stains. For the determination of *A*, *B* and *O* alleles, we examined three nucleotide positions at 261, 796 and 803 on cDNA of the ABO gene. For the deteminatiion of *Se*, *se*1, *se*2 and *se*5 alleles, we examined three nucleotide positions at 385 and 428 on cDNA of SE gene and a SE pseudo gene.

Amplification was accomplished in 25 μL of reaction mixture, which contained 2 ng extracted DNA, 1× PCR buffer (Invitrogen), 1.5 mM $MgCl_2$, 0.2 mM of dNTPs, and 1.25 U of Platinum Taq polymerase (Invitrogen). Primers used for PCR are shown in Table 1. Amplification proceeded as follows: Initial denaturation for 2 min at 94 °C, and then 30 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 65 °C and extension for 2 min at 72 °C. The final extension proceeded for 30 min at 68 °C. The PCR products were analyzed using the ABI PRISM® 3130 Genetic Analyzer and Gene Mapper Software (Applied Biosystems).

3. Results and discussion

Genotyping was performed using fragment analysis of multiplex PCR products. Fig. 1 shows typical pattern of simultaneous detection of ABO and SE blood groupings by fragment analysis. A control peak at 124 bp was always generated at exon 7 (Ex7 cont) in the ABO group reactions through pairing of the S1 and 6FAM-S5 primers. Similarly, a control peak is always generated at 419 bp for SE in the *Se*, *se*1

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doi:10.1016/j.fsigss.2007.10.141

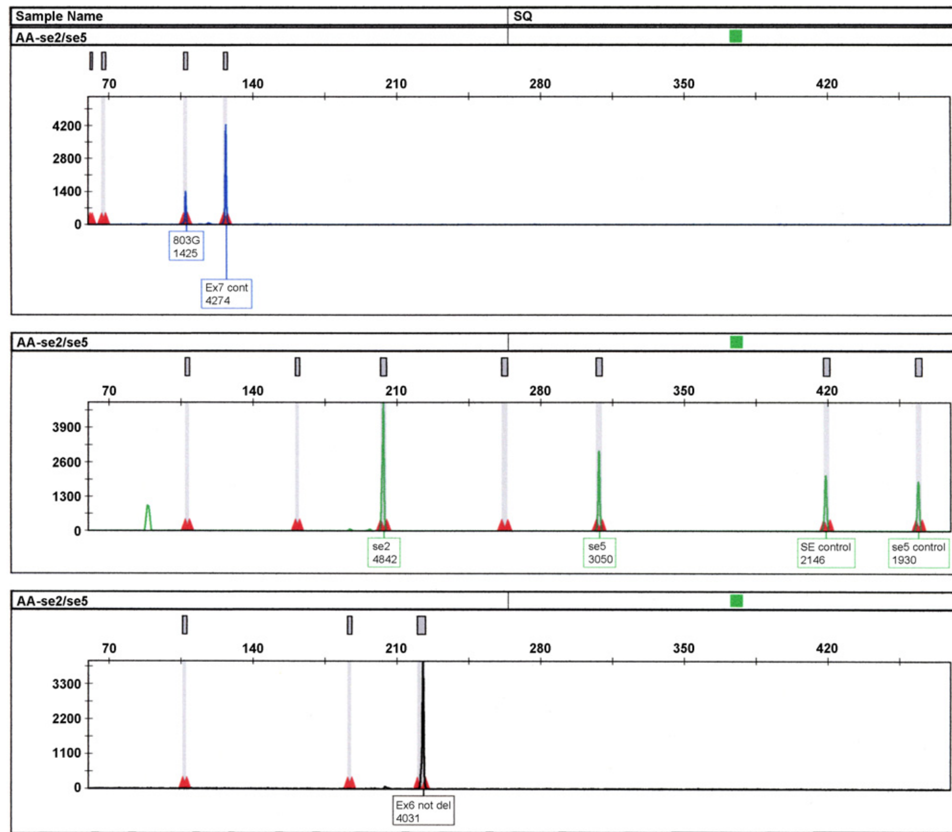

Fig. 1. Fragment chart of PCR products obtained from AA-se2se5 phenotype sample.Seven-fragment peaks (803G, Ex7 cont, se2, se5, SE control, se5 control, and Ex6 not del) were observed in this sample.

Table 1
Primer pairs for simultaneous PCR

Primer	Sequence	[a]FC (pmol)
For ABO		
K1	tail-CAGCTCCATGTGACCGCACGC	2
[b]NED-K2	**NED**-TCGCCACTGCCTGGGTCTCTACC	2
S1	tail-AGCCGGGAGGCCTTCACCTA	2
[b]6FAM-S5	**6FAM**-TGAGCCGCTGCACCTCTTGCA	2
S20	tail-GACGAGGGCGATTTCTACTAGAT	10
[b]6FAM-S60	**6FAM**-ACCGACCCCCCGAAGAATCC	10
For SE		
Fut2-Z1	tail-TCAGGGGGATGTGGACGATCA	5
Fus-T1F	tail-TGGGCATACTCAGCCCGTGT	5
[b]VIC-Fut2-T2R	**VIC**-CGGACGTACTCCCCCGGGAT	5
Fut2-NA1F	tail-GGAGGAGGAATACCGCCACT	5
Fut2-se1F(m7)	tail-ACCGGCTACCCCTGCTCCAA	5
[b]VIC-Fut2-Z4R	**VIC**-ATGGACCCCTACAAAGGTGCC	5

[a] Final concentration for PCR.
[b] Dye-labelled primer.

and *se*2 genotype reactions through pairing of the Fut2-Z1 and VIC-Fut2-Z4R primers, and a control peak at 465 bp for se5 is always generated in the *se*5 genotyping reaction through pairing of the Fus-T1F and VIC-Fut2-Z4R primers. We correctly typed the ABO genotypes, *AA*, *AO*, *BB*, *BO*, *AB* and *OO*, and the SE genotypes, *SeSe*, *Sese*2, *Sese*5, *se*2 *se*2 and *se*2 *se*5. We were not able to find *se*1 non-functional alleles among our Japanese samples. These results corresponded to the serological determination.

This study is the first to simultaneously detect ABO and SE blood groups using a PCR-based strategy. Our method is a simple, rapid and reproducible tool for determining ABO and SE blood groups in forensic biological samples.

Serological determinations are also recommended for typing both ABO and SE blood groups as a complementary test. We are proceeding with comparative studies to standardize our technical system.

Conflict of interest

None.

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