Rapid ABO genotyping directly from fresh blood, hair and stains of blood and buccal epithelial cells

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Introduction

ABO genotyping of forensic specimens is of great value when serological test is not available, e.g., for identifying a decomposed body and blood group typing using body fluids of the nonsecretor. In addition, if we could improve the PCR protocols of molecular genotyping methods which are often time-consuming and call for an initial DNA isolation step that requires the use of expensive kits or reagents, ABO genotyping will be used much more effectively with ease and robustness. Here we describe a rapid and robust ABO genotyping method directly using fresh blood, hair and body fluid stains without prior DNA extraction. Using a fast PCR instrument and an optimized polymerase, the genotyping method—which employs a multiplex allele-specific primer set—can be performed in around 70 min from sample collection to allele designation.

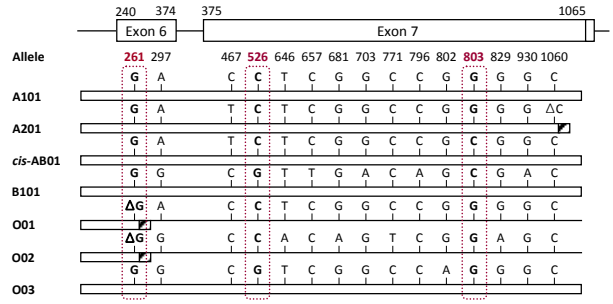
Materials and Methods

Samples

One hundred genomic DNA samples with known ABO sequences were tested. Serially diluted DNA samples (1 ng, 500 pg, 250 pg, 125 pg, 60 pg and 30 pg) of 9947A standard DNA (Promega, Madison, MA, USA) were used to detect sensitivity of a multiplex allele-specific PCR. Fresh blood, hair and buccal swab samples were obtained from 19 voluntary donors. All individuals gave their informed consent to participate, and the study protocol was approved by the institution's ethics committee. Blood and saliva stains were prepared by spotting the blood and by smearing buccal swab onto a FTA® Indicating Classic Card (Whatman, Florham Park, NJ, USA)

Design of a multiplex allele-specific PCR for ABO genotyping

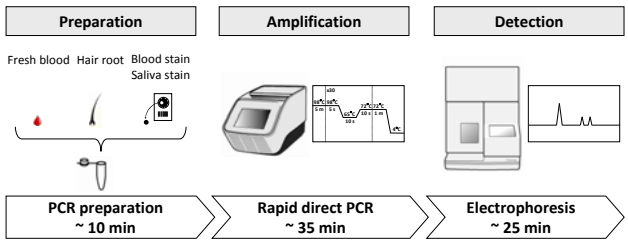
Selection of SNPs from ABO locus



Design of allele-specific PCR primers for three SNPs

Two allele-specific primers and a shared fluorescence-labeled primer were designed for each SNP (nt261, 526 and 803) using the BatchPrimer3 program (http://wheat.pw.usda.gov/demos/BatchPrimer3/). The 3′ end of an allele-specific primer was specific to one of two alleles of a SNP, and an additional mismatch was included at the third or other position from the 3′ end in order to enhance the specificity in the allele-specific PCR reaction. One of the two allele-specific primers was modified to have a tail at its 5′ end, thereby allowing different alleles to produce amplicons of different sizes.

Schematic of rapid direct PCR protocol for ABO blood typing



Rapid direct PCR for ABO genotyping

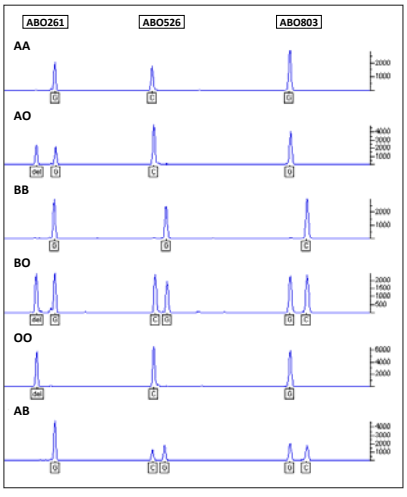
PCR reaction: One μl of fresh blood or a hair root was directly placed in the PCR reaction mixture. A 1.2-mm-diameter punch of blood or saliva stain was used after washing twice with 20 μl of water at 50˚C for 3 min. Total 20 μl reaction contained 4.0 μl of 5X Phire Reaction buffer (Finnzymes, Espoo, Finland), 2.5 U of Phire Hot Start DNA Polymerase (Finnzymes), each dNTPs at 200 μM and each primer at appropriate concentration.

Cycling condition: 98˚C 5 min; 30 cycles of 98˚C 5 sec, 65˚C 10 sec, 72˚C 10 sec; 72˚C 1 min using a Veriti 96-well thermal cycler (Appled Biosystems, Foster City, CA, USA)

Detection system: ABI prism 310 Genetic Analyzer, GeneScan software 3.7 and Genotyper 3.7 software (Applied Biosystems)

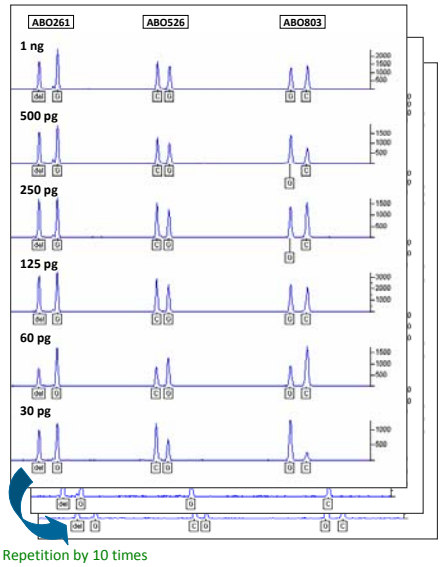
Results

Fig. 1. Electropherograms of six common ABO genotypes in a Korean population



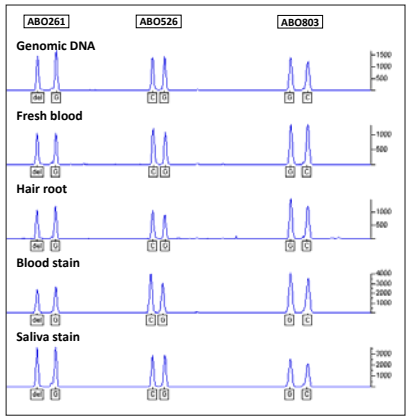
No difference was shown between the ABO genotyping results from multiplex allele-specific PCR and sequencing.

Fig. 2. Sensitivity test with serially diluted 9947A standard DNA



Reliable results were obtained using as low as 60 pg of DNA with no allelic drop-in or drop-out.

Fig. 3. Electropherograms of rapid direct PCR without DNA extraction in BO blood type



Genomic DNA, fresh blood, hair root, blood stain and saliva stain showed good heterozygote peak height ratios and no locus drop-out in rapid direct PCR of all samples from 19 volunteers.

Conclusion

- **The multiplex allele-specific PCR worked very well for all 100 genomic DNA samples, demonstrating sensitivity and accuracy in identifying five alleles (A, B, O01/O02, O03 and cis-AB01).**
- **Sensitivity study for multiplex allele-specific PCR showed good performance in as low as 60 pg of DNA without any pseudo-positive result or allelic drop-out.**
- **Rapid direct PCR for ABO genotyping, which utilizes a fast PCR instrument and an optimized polymerase, enables the direct characterization of blood, hair, and saliva samples without prior DNA extraction in around 70 min using just 1 μl of blood, one hair root, or a 1.2-mm-diameter punch of blood or saliva stain.**
- **The ABO genotyping method demonstrates an example of rapid direct genotyping of SNPs that offers the advantage of time- and cost-efficiency, convenience and reduced contamination during DNA typing analysis.**