Polymerase chain reaction with sequence-specific primers-based genotyping of the human Dombrock blood group DO1 and DO2 alleles and the DO gene frequencies in Chinese blood donors

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The Dombrock blood group system (ISBT 014, DO) was discovered 36 years ago and has been associated with haemolytic transfusion reactions [1,2]. Two common antigens, DO1 (Doα) and DO2 (Doβ), and other three high-incidence antigens – DO3 (Gya), DO4 (Hy) and DO5 (Joα) – were identified using serological methods [1,3–5]. Usually it is difficult to obtain monospecific DO-typing reagents and there are only limited DO gene-frequency studies, especially in the Chinese population [2,6]. As serological DO typing has severe limitations, establishing a DNA-based DO genotyping technique appears to be essential. Recently in a linkage study the DO locus was assigned to chromosome 12p12.3-p13.2 (chromosome 12, short arm, region 1, band 2, sub-band 3, through band 3, sub-band 2) [7]. More recently, the DO gene has been successfully cloned, ending a long period of searching for the molecular basis of the DO1/DO2 polymorphism [8]. Homology studies suggested that the DO molecule is a member of the adenosine 5’-diphosphate (ADP)-ribosyltransferase ecto-enzyme gene family [8]. DO1 and DO2 alleles are the result of a single nucleotide substitution causing an amino acid change within an encoded arginine–glycine–aspartic acid (RGD) motif of the molecule [8]. On the basis of these findings, we have developed, for the first time, a polymerase chain reaction with sequence-specific primers (PCR–SSP)-based DO1 and DO2 genotyping method using newly designed allele-specific primers.

Table 1 Primers for DO typing and amplification of DO exon 2

| Detection of: | Primera | Nucleotide sequence (5’–3’) | Positionb | Primer mix | PCR product size (bp) |
|---------------|---------|-----------------------------|-----------|------------|-----------------------|
| DO1           | DO1R    | TGAATCAACTCTGACAGAATT       | 51210 to 51230 | DOF/DO1R   | 162                   |
| DO2           | DO2R    | GACCTCAACTCTGACAGA TT       | 51210 to 51230 | DOF/DO2R   | 161                   |
| DO1/DO2 alleles | DOF     | CAGGAGTTTGGGAACCAGA AC      | 51371 to 51382 | DOF/DO1R   | 162                   |
| DO exon 2     | 51979F  | GTTCCAAAGAAGAGACCTACC       | 51979 to 51958 | DOF/DO1R   | 162                   |
| 51122R        | GACCCAGTGTCTGCTGACCTG       | 51122 to 51142 | DOF/DO2R   | 161                   |
| HGF           | HGF     | GCCCTCCACCATCCTCCCTA        | 893 to 913 | HGF       | 427                   |
| HGR           | HGR     | TACCAGATTTCCTGTGTTGTTCA     | 1319 to 1298 | HGR       | 427                   |

aF and R indicate forward and reverse primers, respectively.
bNumbering of DO was according to a sequenced BAC clone (GenBank acc. no.: AC007655); HGF numbering was according to clone HGF-N (GenBank acc. no.: M13438).
The complete validity of this method was verified by sequencing analysis. In addition, the concordance rate of 100% was observed. In addition, the sequences (data not shown), further supporting the validity of this typing method.

In conclusion, here we described a simple, accurate and inexpensive method of DO genotyping, which does not require the additional steps of probe hybridization or restriction enzyme digestion. The typing results can be visualized on a single photograph within 3 h, making this reliable method suitable for large-scale typing of potential blood donors without serological backup.

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