ABO GENOTYPING BY PCR-DIRECT SEQUENCING METHOD

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A method of ABO genotyping by PCR-direct sequencing was developed. The method includes two steps. First, two fragments from 233bp-433bp and 660bp-788bp regions of a transferase gene were amplified respectively by using 261st primers and 703rd primers. The 261st primer sequences are, forward 5' ACACC GTGGA AGGAT GTCCTC 3' and reverse 5' AATGT CCACA GTCAC TCGCC 3'. The 703rd primer sequences are forward 5' TGGAG ATCCT GACTC CGCTG 3' and reverse 5' GTAGA AATCG CCCTC GTCCTT 3'. Second, using two reverse primers from 261st and 703rd primers with ABI Prism® BIGDYE Terminator™ Cycle Sequencing Kit, the sequences of two fragments from 233bp-433bp and 660bp-788bp regions were analyzed. One hundred bloodstains whose ABO genotypes were already determined by Amp-RFLP method beforehand were analyzed in our study by this method. The results showed that two nucleotide substitutions at 261st and 297th were found in 233bp-433bp region, and a nucleotide substitution at 703rd was found in 660bp-788bp region. At 261st, the nucleotide was guanine in A and B alleles, and ademine in O allele. At 297th, the nucleotide substitution existed between A and B alleles. As this position, O allele was subdivided into two types, O^A and O^G. At 703rd, the nucleotide was guanine in A and O alleles, and ademine in B allele. Therefore, 10 genotypes, AA, AO^A, AO^G, AB, BB, BO^A, BO^G, O^AO^A, O^GO^G, and O^AO^G, could be clearly determined by analyzing the 233bp-433bp and 660bp-788bp regions. The technique of PCR-direct sequencing provides an effective and new method for ABO genotyping further.