Four-Color Multiplex PCR Assay for the Simultaneous Detection of Four Allelic Variants in a Closed Tube Using a Single Thermal Cycler Program on the iCycler IQ™ Real-Time PCR Detection System

Abstract

Homogeneous allele-specific assays for single nucleotide polymorphism (SNP) detection have several advantages over the more traditional techniques based on the use of the polymerase chain reaction (PCR). For instance, labor is significantly reduced because the automated handling of sample preparation and PCR is less time-consuming than traditional techniques. Moreover, homogeneous allele-specific assays are less labor-intensive because they can be performed in microtiter plates, allowing for high-throughput screening of genetic variations in large populations. In this study, we developed a real-time multiplex assay for the simultaneous detection of four allelic variants in a single thermal cycle using a single thermal cycler program on the iCycler IQ™ Real-Time PCR Detection System. The assay was designed to detect the most common hemoglobinopathies and hepcidinopathies, including the FVL and PT G20210A mutations, using a four-color multiplex PCR reaction. The results generated by the multiplex four-color assay were compared with those obtained with a reference method, and the genotyping results were analyzed by the software. The assay was found to be reliable, accurate, and sensitive, with a high degree of specificity and reproducibility. The assay was also shown to be cost-effective and time-saving, making it an ideal tool for genetic screening and diagnosis.
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