Multiplex assay for the detection of common Mediterranean beta-thalassemia mutations

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INTRODUCTION

Hemoglobinopathies are the most abundant group of genetic abnormalities in humans, caused by genetic defects affecting the globin genes. Several *HBB* gene mutations occur at high frequencies in the Mediterranean region: IVS-I-110 (G->A), IVS-I-1 (G->A), IVS-I-6 (T->C), Codon 39 (C->T), IVS-II-745 (C->G), Codon 5 (-CT); CCT(Pro)->C--, Codon 6 (-A); GAG(Glu)->G-G, Codon 8 (-AA); AAG(Lys)->--G, and beta6(A3) Glu>Val (Figure 1). We set out to develop a reliable, rapid and cost-effective molecular diagnostic assay targeting these mutations.

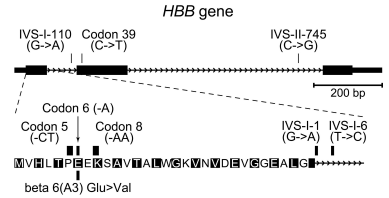

Figure 1. Common Mediterranean beta-thalassaemia and HbS mutations

MATERIALS AND METHODS

A 1.8 kb *HBB* fragment encompassing all mutations included in the assay was amplified by PCR. The purified PCR product was directly used as template in a primer extension reaction containing a mutation-specific primer cocktail. Extension products were separated by capillary electrophoresis. The majority of the mutations were detected on both genomic strands (Figure 2).

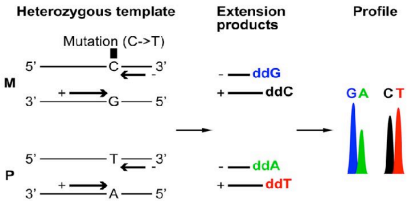

Figure 2. Schematic representation of the single-nucleotide primer extension assay

RESULTS AND DISCUSSION

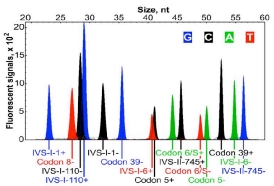

Figure 3. Electropherogram of the normal *HBB* sequence

We optimized the extension primer cocktail using normal DNA samples (Figure 3). The ability of the cocktail to detect the assayed mutations was demonstrated on a set of heterozygous samples (Figure 4). Finally, the test was validated using >100 reference chromosomes (Figure 5). These analyses showed that the new assay detects the assayed mutations with high sensitivity and specificity.

The novel assay utilizes both strands for mutation interrogation reaching very high levels of accuracy, equivalent to sequencing both genomic strands. The new primer extension assay can be applied across wide geographic areas meeting the highest diagnostic standards.

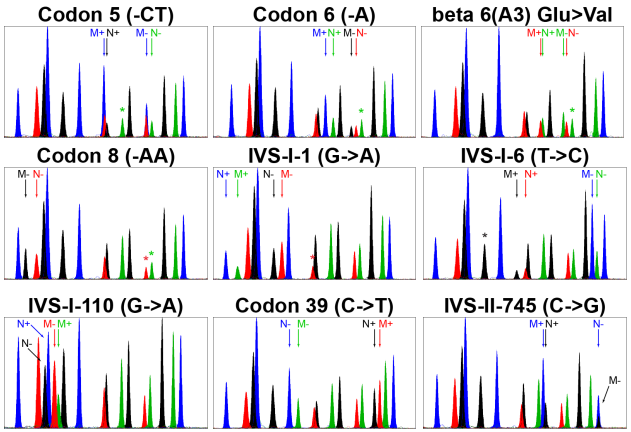

Figure 4. Electropherogram of the heterozygous samples

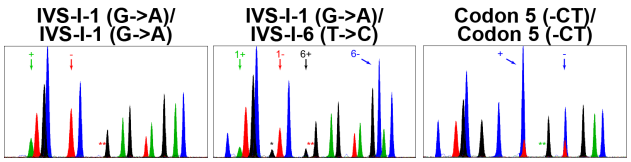

Figure 5. Electropherogram of the homozygous samples

ACKNOWLEDGMENTS

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