OP 02 - Designing and implementation of a novel PCR assay for detection of Hb adana genetic variant associated with thalassemia disorder

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Introduction: Thalassemia is inherited as an autosomal recessive disorder. The severity of the thalassemia disorder depends on the nature of the genetic variant. The Main recognized molecular basis for the disorder is SNV's and gene deletions. Hb Adana genetic variant is one such point mutation occurring in the HBA gene. The objective of the study was to design and implement a novel PCR assay for detection of Hb Adana (HBA2:c.179 G>A; rs28928878) genetic variant associated with thalassemia.

Methods: A tetra-amplification refractory mutation system polymerase chain reaction (T-ARMS PCR) method was designed for the HBA2:c.179 G>A (rs28928878) genetic variant. The designed T-ARMS PCR method was then optimized by performing a gradient PCR. Implementation of the optimized protocol was done, using the DNA of venous blood samples, that were obtained from a cohort of patients with beta-thalassemia who had given prior informed consent for such studies. Twelve venous blood samples were genotyped for this study to verify the novel PCR assay. Verification of results was done by sanger validation.

Results: The expected results were obtained by the T-ARMS PCR assay. The bands that were obtained through the gel images, were corresponding accurately with expected base pair lengths and were specific. Major allele(G) frequency for HBA2:c.179 G>A was 100% and the minor allele (A) was not detected in the sample cohort. Genotypic frequencies for HBA2:c.179 G>A genetic variants, GG was 100% (12/12) and GA was 0% (0/12).

Conclusions: Designing, optimization and implementation of the tetra-amplification refractory mutation system polymerase chain reaction (T-ARMS PCR), as a novel PCR assay was successful. This novel PCR assay can be used to detect the presence of HBA2:c.179 G>A genetic variants in patients with thalassemia.

Keywords: Tetra-ARMS PCR, *HB Adana*, Thalassemia

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