

# Design and Implementation of an assay for genetic variants associated with Non deletion Alpha Thalassemia in a cohort of Sri Lankan population

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## Background & Objectives

Non-deletion variants are a rare cause of alpha thalassaemia. These Non-deletional variants of alpha thalassemia includes missense and point variants that alters the genomic regions of the  $\alpha$ -globin genes that are critical for the normal expression. *Hb Quong Sze* and *Hb Adana* were found to be the commonest types of non-deletion alpha thalassemia within the South East Asian population. The present study was undertaken to design an allele specific PCR of selected non-deletion variants and to detect the presence of these novel variants in Sri Lankan population.

## Method (s) and Results

Novel single variant tetra primer-amplification refractory mutation system (T-ARMS) polymerase chain reaction (PCR) assays were designed for the *HBA2:c.377 T>C* (rs41397847) and *HBA2:c.179 G>A* (rs28928878) variants. The optimum annealing temperatures, where 61.8<sup>0</sup>C for the *HBA2:c.377 T>C* and 68.3<sup>0</sup>C for *HBA2:c.179 G>A*. *HBA2:c.377 T>C* variant was further optimized, validated by Sanger sequencing. Implementation by genotyping for the variants were performed by using an existing blood sample collection. A total of 100 samples were genotyped.

Among the 100 samples genotyped, the genotype frequency for the homozygous variant (C/C) was 0.88%, heterozygotes (T/C) frequency was of 0.04% and homozygous wild type (T/T) was 0.08%. The respective variant allele frequency was (0.1%) and ancestral allele frequency was (0.9%). Out of the 100 samples genotyped, no variant allele samples were detected for this variant. The allele frequency was 0% respectively.

## Conclusion

The *HBA2:c.377 T>C* variant was optimized and implemented. T-ARMS PCR was used to Genotype the HBA2  $\alpha$ -globin gene. The minor alleles of the *HBA2:c.377 T>C* variant were identified in the genotyped cohort in the heterozygous form. Optimization on *HBA2:c.179 G>A* for non-deletion alpha thalassemic patients' needs to be further developed. T-ARMS PCR can be used as a low-cost assay for detecting non-deletion alpha thalassemic variants.

Keywords: T-ARMS PCR, Non-deletion Alpha-thalassemia, *HBA2:c.377 T>C*, *HBA2:c.179 G>A*,

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