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# ABSTRACT BOOK



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## In-Silico Design of Multiplex PCR Primers for Nanopore-Based Detection of Thalassaemia Mutations in Malaysian Population

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### ABSTRACT

**Introduction:** Thalassaemia is a common hereditary disorder in Malaysia, with a carrier rate of 6.8%. Thalassaemia has significant genetic heterogeneity in both alpha and beta forms, and between different race groups. Standard molecular testing often targets only common mutations, hence missing rare variants. A multiplex PCR approach integrated with long-read sequencing offers a promising solution. This study aimed to design primer sets capable of amplifying all regions of the *HBA1/A2*, *HBB*, *HBD* and *HBG1/G2* regions for comprehensive thalassaemia detection. **Materials and methods:** A literature review of published studies on the molecular epidemiology of thalassaemia in Southeast Asia was conducted to identify prevalent and clinically significant mutations. Primer sets were designed using Primer3 and IDT PrimerQuest, targeting regions within *HBA1/A2*, *HBB*, *HBD* and *HBG1/G2* genes. Amplicons were optimised for size (5000 to 10000 bp) to ensure compatibility with Nanopore sequencing. **Results:** Seven primer pairs were designed to cover both deletional and non-deletional variants, including common mutations such as Hb Constant Spring, CD26 (HbE), IVS1-5, and CD41/42 and also common deletions such as --SEA,  $-\alpha^{3.7}$  and Filipino  $\beta^0$  deletion. Additionally, primer pairs that flank rare deletions, including alpha Zero ( $\alpha^0$ ) deletion (--GB) were designed. All primer sets were evaluated in silico for melting temperature, specificity, GC content, and amplicon overlap. **Conclusion:** This study presents a mutation-informed primer design strategy for a multiplex PCR assay tailored to the genetic landscape of thalassaemia in Malaysia. By incorporating both common and rare mutations, the primer panel offers a foundation for long-read sequencing of the haemoglobin genes.

**Keywords:** Multiplex PCR; nanopore sequencing; primer design; thalassaemia