

Optimization and Validation of Tetra Primer-Amplification Refractory Mutation System-based PCR for Detecting Rare Pathogenic *BRCA1* deletions

Kavinya Rajan^{1,2}, Dr. Kawmadi. W. Gunawardena³

¹*Centre for Genetics and Genomics, Faculty of Medicine, University of Colombo, Sri Lanka*

²*Faculty of Life and Medical Sciences, BMS Campus*

³*Oxford University Hospitals NHS Foundation Trust, United Kingdom*

Introduction - Breast Cancer (*BRCA*) genes encode for DNA damage repair proteins. *BRCA* gene mutations have been reported in numerous inherited and sporadic cancers, particularly breast cancer. The presence of pathogenic variants significantly increases cancer susceptibility. However, most mutations remain underdiagnosed in Sri Lanka's resource-limited settings due to the high cost of sequencing. Hence, this study was designed to develop, optimize and validate a Tetra Primer-Amplification Refractory Mutation System (T-ARMS PCR)-based PCR assay for the detection of three rare, pathogenic *BRCA1* deletions.

Methods - Three under-reported pathogenic *BRCA1* deletion variants (c.237del, c.5289del and c.1575del), that were previously observed in Sri Lankan patients, were selected for this study. Two pairs of primers (wild-type specific forward inner, mutant-specific reverse inner, forward outer and reverse outer) were designed. Inner primers were designed manually, while Primer3 software was used for outer primers. The mutant-specific primers were designed by incorporating the mutation at their 3'-end. Primer specificity and other parameters were verified. The annealing temperature and concentration of primers and magnesium chloride were optimized. T-ARMS PCR was performed to screen for the selected variants using DNA extracted from 44 breast cancer patients' samples. Results were validated through Sanger sequence.

Results - Among the 44 samples tested, only one heterozygous carrier for the c.237del variant was identified, while all others were wild-type for the three loci. Similar results were also indicated in the chromatograms obtained through sequencing.

Discussion - Generation of clear bands of expected size indicated the successful optimization and validation of the T-ARMS PCR assay for detecting selected variants. The absence of c.1575del and c.5289del mutations suggests the rarity of these variants. Validation through Sanger sequencing confirmed the reliability of the results. In conclusion, the optimized T-ARMS PCR provides a rapid and cost-effective approach for screening *BRCA1* deletion variants, making it suitable for use in resource-limited diagnostic settings.

Keywords - *BRCA1*, Breast cancer, Deletion variant, Pathogenic, T-ARMS PCR