

## Optimization of Polymerase Chain Reaction Condition to Amplify Exon 9 of PIK3CA Gene in Preventing False Positive Detection Caused by Pseudogene Existence in Breast Cancer

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**Abstract :** Breast cancer is a regulated by many genes. Defect in PIK3CA gene especially at position of exon 9 (E542K and E545K), called hot spot mutation induce early transformation of breast cells. The early detection of breast cancer based on mutation profile of this hot spot region would be hampered by the existence of pseudogene, marked by its substitution mutation at base 1658 (E545A) and deletion at 1659 that have been previously proven in several cancers. To the best of the authors' knowledge, until recently no studies have been reported about pseudogene phenomenon in breast cancer. Here, we reported PCR optimization to to obtain true exon 9 of PIK3CA gene from its pseudogene hence increasing the validity of data. Material and methods: two genomic DNA with Dev and En code were used in this experiment. Two pairs of primer were design for Standard PCR method. The size of PCR products for each primer is 200bp and 400bp. While other primer was designed for Nested-PCR followed with DNA sequencing method. For Nested-PCR, we optimized the annealing temperature in first and second run of PCR, and the PCR cycle for first run PCR (15x versus 25x). Result: standard PCR using both primer pairs designed is failed to detect the true PIK3CA gene, appearing a substitution mutation at 1658 and deletion at 1659 of PCR product in sequence chromatogram indicated pseudogene. Meanwhile, Nested-PCR with optimum condition (annealing temperature for the first round at 55oC, annealing temperaturung for the second round at 60,7oC with 15x PCR cycles) and could detect the true PIK3CA gene. Dev sample were identified as WT while En sample contain one substitution mutation at position 545 of exon 9, indicating amino acid changing from E to K. For the conclusion, pseudogene also exists in breast cancer and the appllication of optimazed Nested-PCR in this study could detect the true exon 9 of PIK3CA gene.

**Keywords :** breast cancer, exon 9, hotspot mutation, PIK3CA, pseudogene

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