

Development of sybr green i-based melting curve method for HER2I655V polymorphism detection in breast cancer

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Abstrak

Background: Currently available molecular method to detect HER2I655V polymorphism such as PCR-RFLP is hampered by costly experimental method and post-PCR treatment requirement that make this technique is not meeting for high-throughput analysis purpose. In this study we developed a accurate, simple, low cost and rapid test to detect polymorphism at HER2 gene using SBR Green I based-melting curve method. Methods: Two forward allele-specific primers and one common reverse primer were used then these primers were tested to discriminate known genotypes of genomic templates (GG type or AA type) and genomic samples retrieved from breast cancer patients. Results: Melting curve analysis derived from SYBR Green I-based allele-specific PCR with defined primers concentration and annealing temperature at 54.3 °C showed good discrimination level of T_m peaks in which GG genotype melted at 89 °C slightly higher than AA genotype which melted at 86 °C, while AG genotype harbored both of homozygous T_m characteristics. Conclusions: This preliminary result will be as basic for further large-scale typing of HER2I655V polymorphism.