Optimalisasi Metode Tm *Shift* SYBR *Green* I *Real Time* PCR untuk *Genotyping* SNP Ile655Val HER-2 Pasien Kanker Payudara

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Intisari

SNP Ile655Val HER-2 diketahui sebagai molekular marker keganasan kanker payudara dengan faktor prognostik yang buruk. Beberapa metode *genotyping* yang digunakan untuk menganalisis SNP meliputi; PCR RFLP, AS PCR, PCR *seq* dan *Real Time* PCR dengan label probe. Masing-masing metode tersebut memiliki kelebihan dan kekurangan dalam hal teknis operasional dan biaya yang dikeluarkan. Penelitian ini bertujuan untuk mengkarakterisasi kit diagnostik SNP Ile655Val HER-2 yaitu Tm *Shift* SYBR *Green I Real Time* PCR untuk membedakan variasi alel SNP Ile655Val berdasarkan perbedaan pola *melting peak* dan *temperature melting* (Tm). Kit diagnostik tersebut dikarakterisasi berdasarkan reprodusibilitas, sensitifitas dan stabilitas. Berdasarkan hasil yang diperoleh, Tm *Shift* SYBR *Green* I *Real Time* PCR mampu membedakan SNP Homozigot dan Heterozigot maupun *wild type*. *Range* Tm untuk SNP HER-2 GG dan WT (AA) adalah 85 ± 0,14^{0}C, 82,5 ± 0,23^{0}C, kemudian nilai Ct masing maisng alel GG, AG dan AA yaitu 19,6 ± 0,27; 22,5 ± 0,23; dan 18,6 ± 0,22. Reprodusibilitas metode dianalisis menggunakan Kappa statistik menghasilkan koefisien Kappa sebesar 1 yang menunjukkan kesesuaian metode dengan PCR *seq* sebagai *gold standard method*. Metode Tm *Shift* SYBR *Green* I *Real Time* PCR memiliki sensitifitas cukup baik karena mampu mengamplifikasi sampel genotipe GG dengan seksama di dalam sampel yang diformulasi dengan sampel genotipe AA. Hasil menunjukkan nilai Ct di *range* GG pada semua formulasi namun *melt peak* terbentuk yang mencirikan alel AA. Disimpulkan bahwa metode Tm *Shift* SYBR *Green* I *Real Time* PCR cepat, mudah, reprodusibel, sensitif dan stabil sebagai kit diagnostik untuk *genotyping* SNP Ile655Val HER-2.

Kata Kunci : Tm *Shift* SYBR *Green* I *Real Time* PCR; SNP Ile655Val HER-2 ; Kanker Payudara

Optimalization Methods of Tm *Shift* SYBR *Green* I Real Time PCR for SNP *Genotyping* Ile655Val HER- 2 Breast Cancer Patients

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Abstract

SNP Ile655Val HER-2 is known as a molecular marker of malignancy of breast cancer with poor prognostic factors. Several *genotyping* methods used to analyze SNPs include; PCR RFLP, AS PCR, PCR seq and Real Time PCR probe labelled. Each of these methods has its advantages and disadvantages in terms of technical, operational and cost. This research aims to characterize a diagnostic kit SNP Ile655Val HER-2 that is Tm *Shift* SYBR *Green* I Real Time PCR to distinguish the SNP allele variation Ile655Val based on different Ct value and the melting *peak* melting temperature (Tm). The diagnostic kit is characterized by reproducibility, sensitivity and stability. Based on the results obtained, Tm *Shift* SYBR *Green* I Real Time PCR is able to distinguish the SNP Homozygous and Heterozygous or *wild type*. *Range* Tm for SNP HER-2 GG and WT (AA) were 85 ± $0{,}14^0$C, 82.5 ± $0{,}23^0$C, the value of each allele Ct GG, AG and AA were 19.6 ± 0.27; 22.5 ± 0.23; and 18.6 ± 0.22. The reproducibility of method were analyzed by Kappa statistic resulting in 1 KAPPA coefficient , which indicates conformance with the PCR method as the gold standard method PCR seq. Tm *Shift* SYBR *Green* I Real Time PCR method were sensitive due to the ability to amplify samples of GG genotype sample precisely which formulated with the AA genotype samples. The results show the Ct value in the *range* GG in all formulations but *melt peak* form characterizes the alleles AA. It was concluded that the method Tm *Shift* SYBR *Green* I Real Time PCR is fast, easy, reproducible, sensitive and stable as a diagnostic kit for SNP *genotyping* Ile655Val HER-2.

Keywords : Tm *Shift* SYBR *Green* I *Real Time* PCR; SNP Ile655Val HER-2 ; Breast cancer