Optimasi Primer Gen Cytochrome b (Cytb) untuk Mendeteksi Babi Domestik (Sus scrofa domesticus) dan Babi Hutan (Sus scrofa) Menggunakan Metode qPCR sebagai Pengembangan Halal Kit = Optimization Primer of the Cytochrome b (Cytb) Gene for Detecting Domestic Pig (Sus scrofa domesticus) and Wild Boar (Sus scrofa) Using qPCR Method as Halal Kit Development

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Abstrak

Perkembangan teknologi menyebabkan praktik pencampuran produk makanan menggunakan substansi non-halal. Dokumen (International Standardization Organization/ Technical Specification) ISO/TS 20224-3: 2020 digunakan sebagai prosedur standar uji deteksi kehalalan berbasis Deoxyribonucleic Acid (DNA) memanfaatkan metode Quantitative Polymerase Chain Reaction (qPCR) yang berlaku secara internasional melalui gen Beta actin (ACTB) sebagai gen target. Namun, kemampuan gen ACTB sebagai gen target belum banyak teruji langsung melalui reaksi PCR sehingga dibutuhkan evaluasi potensi gen target alternatif lain melalui optimasi primer seperti gen Cytochrome b (Cytb) untuk mendeteksi kandungan babi domestik (Sus scrofa domesticus) dan babi hutan (Sus scrofa). Metode yang digunakan terdiri atas desain primer dan probe, optimasi suhu annealing primer dan probe, uji spesifisitas in silico, uji sensitivitas in vitro, serta pengolahan dan analisis data. Adapun sampel yang digunakan untuk uji sensitivitas in vitro adalah pig genomic DNA. Berdasarkan pengujian yang dilakukan, primer dan probe gen Cytb memiliki suhu annealing optimal pada suhu 55°C. Uji spesifisitas in silico membuktikan bahwa sekuens primer dan probe gen Cytb memiliki kemampuan deteksi pada sekuens babi domestik dan babi hutan. Uji sensitivitas menggunakan qPCR pada gen ACTB membentuk kurva standar dengan nilai y=-3,6541x +38,385 dan R2=0,9967, serta LoD sebesar 5 pg/uL. Nilai linearitas (0,9967) dan efisiensi (87,78%) yang dihasilkan masuk ke dalam rentang standar sesuai literatur karena berada 0,98 untuk linearitas dan rentang 80%—120% untuk efisiensi. Sementara itu, uji sensitivitas menggunakan qPCR pada gen Cytb membentuk kurva standar dengan nilai y=-2,7222x + 32,196 dan R2= 0,9867, serta LoD sebesar 1 pg/uL. Nilai linearitas (0,9867) yang dimiliki masuk ke dalam rentang standar, tetapi nilai efisiensi (132,99%) melebihi rentang persentase yang baik akibat kemungkinan konsentrasi serial dilusi yang kurang sesuai dan protokol yang belum optimal. Gen Cytb memiliki jangkauan sensitivitas yang lebih baik dibandingkan gen ACTB. Keseluruhan grafik hasil membentuk kurva sigmoid yang valid sebagai hasil uji qPCR. Oleh karena itu, berdasarkan uji spesifisitas in silico dan sensitivitas in vitro yang dilakukan dapat disimpulkan bahwa gen Cytb berpotensi dijadikan gen target altenatif sebagai pengembangan halal kit.

.....Technological developments have led to the practice of mixing food products using non-halal substances. Document (International Standardization Organization/Technical Specification) ISO/TS 20224-3: 2020 is used as a standard procedure for Deoxyribonucleic Acid (DNA)-based halal detection test that is internationally applicable through the Beta actin gene (ACTB) as the target gene. However, the ability of the ACTB gene as a target gene has not been tested directly through PCR reactions, so it is required to evaluate the potential of other alternative target genes through primer optimization such as the Cytochrome b (Cytb) gene to detect domestic pig (Sus scrofa domesticus) and wild boar (Sus scrofa) containment. The method

used was comprised of primer and probe design, primer and probe annealing temperature optimization, in silico specificity test, in vitro sensitivity test, and data processing and analysis. The sample used for the in vitro sensitivity test is pig genomic DNA. Based on the tests conducted, primers and probes of the Cytb gene have an optimal annealing temperature at 55°C. The in silico specificity test proved that the primer sequences and Cytb gene probes have the ability to detect domestic pig and wild boar sequences. The sensitivity test using qPCR on the ACTB gene forming a standard curve with a value of y=-3.6541x +38.385 and R2=0.9967, and LoD of 5 pg/uL. The linearity (0.9967) and efficiency (87.78%) values generated are in the standard range according to the literature because they are 0.98 for linearity and 80%—120% range for efficiency. Meanwhile, the sensitivity test using qPCR on the Cytb gene is forming a standard curve with a value of y= -2.7222x + 32.196 and R2= 0.9867, and LoD of 1 pg/uL. The linearity value (0.9867) is within the standard range, but the efficiency value (132.99%) exceeds the good percentage range as a result of the possibility of inappropriate serial dilution concentrations and an unoptimal protocol. The Cytb gene has a better sensitivity range than the ACTB gene. The overall result graph forms a sigmoid curve which is valid as a qPCR test result. Therefore, based on the in silico specificity and in vitro sensitivity tests, it can be concluded that the Cytb gene has the potential to be used as an alternative target gene as a halal kit development.