

Pengembangan Metode Deteksi DNA Babi (*Sus scrofa*) Menggunakan Ekstraksi DNA Otomatis Dan Analisis Real-Time Polymerase Chain Reaction (RT-PCR) = The Development of Pig (*Sus scrofa*) DNA Detection Using Automated DNA Extraction and Real-Time Polymerase Chain Reaction (RT-PCR) Analysis

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Abstrak

Penelitian ini mengembangkan metode deteksi spesies babi (*Sus scrofa*) pada sampel daging campuran menggunakan automasi ekstraksi DNA magLEAD gC. DNA dianalisis menggunakan PCR dan TaqMan probe RT-PCR dengan primer spesifik untuk gen Cytochrome c oxidase I (COI), Cytochrome b (Cytb), dan NADH5 dehydrogenase 5 (ND5). Hasil menunjukkan bahwa ekstraksi DNA otomatis menghasilkan konsentrasi DNA 129,4–388,5 ng/L pada daging mentah dan 66,4–89,5 ng/L pada bakso dengan rasio kemurnian A260/A280 dan 260/A230 > 1,8. Primer COI, Cytb dan ND5 dapat mendeteksi DNA babi. PCR dan RT-PCR in vitro menunjukkan ketiga primer hanya mendeteksi DNA babi. Efisiensi amplifikasi RT-PCR primer COI, Cytb, dan ND5 adalah 144,14% (R²=0,982), 88,05% (R²=0,998), dan 81,25% (R²=0,997) dengan batas deteksi 0,0001 ng/L, 0,001 ng/L, dan 0,001 ng/L. Primer/probe Cytb dan ND5 mendeteksi bakso dengan campuran daging babi hingga 0,1% (w/w).

.....This study developed a method to detect pig species (*Sus scrofa*) in mixed meat samples using automated DNA extraction with the magLEAD gC. DNA was analyzed using PCR and TaqMan probe RT-PCR with specific primers for the genes Cytochrome c oxidase I (COI), Cytochrome b (Cytb), and NADH5 dehydrogenase 5 (ND5). Results showed that automated DNA extraction produced DNA concentrations of 129.4–388.5 ng/L in raw meat and 66.4–89.5 ng/L in processed meatballs with purity ratios A260/A280 dan 260/A230 > 1.8. The COI, Cytb and ND5 primers could be used to detect pig DNA. In vitro PCR and RT-PCR showed that all three primers only detected pig DNA. The RT-PCR amplification efficiency for COI, Cytb, and ND5 primers were 144,14% (R²=0,982), 88,05% (R²=0,998), dan 81,25% (R²=0,997) with detection limits of 0.0001 ng/L, 0.001 ng/L, and 0.001 ng/L. The Cytb and ND5 primers/probes detected meatballs with pig meat content as low as 0.1% (w/w).