

**DESAIN DAN UJI KINERJA PROBE TaqMan REAL-TIME POLYMERASE CHAIN REACTION (RT-PCR) SPESIFIK TIKUS HITAM (*Rattus rattus*) DENGAN GEN SITOKROM-B DNA MITOKONDRIA**

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**INTISARI**

Telah dilakukan uji kinerja terhadap *primer forward*, *probe* TaqMan, dan *primer reversed* hasil desain menggunakan *teknik Real-time Polymerase Chain Reaction* (RT-PCR) *probe* TaqMan spesifik tikus hitam berdasarkan urutan gen sitokrom-b DNA mitokondria. Penelitian ini bertujuan untuk mendesain dan mengetahui kinerja *primer* dan *probe* TaqMan yang bersifat spesifik terhadap DNA tikus hitam sebagai metode deteksi kontaminasi daging tikus hitam berbasis DNA.

Penelitian ini dilakukan dengan dua tahap. Tahapan pertama yaitu desain dan sintesis *primer* serta *probe* TaqMan secara *in silico*, tahapan kedua yaitu uji kinerja yang meliputi uji spesifitas menggunakan DNA tikus hitam sebagai kontrol positif dan DNA lima spesies lain (sapi, kambing, kuda, babi, dan anjing) sebagai kontrol negatif, uji presisi dengan pengulangan amplifikasi kontrol positif sebanyak sepuluh kali, uji sensitivitas dengan pengenceran DNA tikus hitam hingga 0,5 pg, dan uji persentase kontaminasi yang dilakukan dengan pencampuran antara DNA tikus hitam dan DNA sapi dengan persentase 1, 2, 5, 10, 25, 50, dan 100 % (v/v).

*Primer forward* (5'-GACTTACTAGGAGACCCAGACA-3'), *probe* TaqMan (5'-ACACACCTGCTAACCCACTAAATACCC-3'), dan *primer reversed* (5'-TGTTAGGGATGGAGCGTAGA-3') dengan ukuran fragmen target 118 pb merupakan *primer* dan *probe* TaqMan terpilih berdasarkan uji kinerjanya secara *in silico*. *Primer* dan *probe* TaqMan terpilih hanya mengamplifikasi DNA tikus hitam sehingga bersifat spesifik. Hasil uji kinerja menunjukkan bahwa metode ini spesifik digunakan untuk mendeteksi cemaran tikus hitam hingga 0,50 pg dan konsentrasi 1 % (v/v). Oleh karena itu, *primer* dan *probe* hasil sintesis dapat digunakan sebagai penanda spesifik berbasis DNA untuk deteksi kontaminasi tikus hitam.

Kata kunci : *Polymerase Chain Reaction*, DNA mitokondria, tikus hitam, *probe* TaqMan spesifik, analisis pada daging.

## **DESIGN AND PERFORMANCE STUDY OF TaqMan PROBE REAL-TIME POLYMERASE CHAIN REACTION (RT-PCR) ASSAY SPECIFIC TO BLACK RAT (*Rattus rattus*) BASED ON CYT-B GENE MITOCHONDRIAL DNA**

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### **ABSTRACT**

A performance test of primer forward, TaqMan probe, and primer reversed as design results have been conducted using TaqMan probe specific real-time polymerase chain reaction (RT-PCR) method specific to black rat derived from cytochrome-b sequence of mitochondrial DNA. The objective of this research was to create primer and TaqMan probe specific to black rat's DNA and discover the performance of primer and TaqMan probe for the DNA based-detection method of black rat contamination.

This research consisted in two stages. Primer and probe TaqMan were designed and synthesized using in silico method in 1<sup>st</sup> stage, while in 2<sup>nd</sup> stage, the performance test of primer and TaqMan probe were conducted. The performance test including specificity test using black rat's DNA as positive control and DNA of five other species (cattle, goat, horse, pig and dog) as negative control, precision test by ten times repetition of positive control amplification. As for sensitivity test, the black rat's DNA was diluted up to 0.5 pg and for contamination percentage test, the DNA of black rat was mixed to beef's DNA with percentage of 1, 2, 5, 10, 25, 50, and 100 % (v/v).

Forward primer (5'-GACTTACTAGGAGACCCAGACA-3'), TaqMan probe (5'-ACACACCTGCTAACCCTAAATACCC-3'), and reversed primer (5'-TGTTAGGGATGGAGCGTAGA-3') with a target size of 118 bp were selected based on its performance. The selected primers and probe only amplify DNA of black rat, but not others DNA, so that they are specific. The performance test results showed that this specific method is used to detect black rat contamination up to 0.5 pg and 1 % (v/v) concentration. Therefore, the synthesized primers and probe can be used as specific marker based on DNA for detection of black rat contamination.

**Keywords :** Polymerase Chain Reaction, mitochondrial DNA, black rat, Taqman-specific probe, meat analysis.



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