

**DESIGN AND PERFORMANCE STUDY OF TaqMan PROBE  
REAL-TIME POLYMERASE CHAIN REACTION ASSAY  
SPESIFIC TO PORK (*Sus scrofa*) BASED ON ATPase 6 GENE**

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**ABSTRACT**

A real-time polymerase chain reaction method using TaqMan probe assay specific to the mitochondrial ATPase6 gene of pork has been conducted. The objective of this research was to design and study the specificity and performance of ATPase 6 TaqMan probe and primers specific to pork (*Sus scrofa*) DNA as testing method for detection of pork DNA in meat-based products.

TaqMan probes and primers were designed using PrimerQuest tool and the homology of the assay was tested using BLAST. The method performance test was divided into specificity test by testing the assay to pork, dog, rat, beef, chicken, goat, and horse DNA; precision test by amplifying 10 positive controls; and the limit of detection determination by amplifying a serially diluted pork DNA template from 50 ng to 5 pg and amplifying binary mixture of pork and beef DNA with pork contamination of 1, 2, 5, 10 25, 50, and 100%.

The forward primer (5'-CACACCCACCCACACAACCTAT-3') and reverse primers (5'-GGTAGAAAGTGGGCTAGTGATG-3'), associated with probe (5'-/FAM/TCAGCAACC/ZEN/GTATTCACAGGATTCCG/3IABkFQ/-3'), targeting the pork ATPase 6 gene region, was obtained as the designed results. The real-time PCR detection method using designed specific TaqMan probes and primer has ability to specifically differentiate pork DNA. The method showed good precision and highly sensitive to detect up to 5 pg of pork DNA and 1% of pork contamination in mixtures.

Keywords: meat analysis, TaqMan probe, real-time PCR, ATPase 6 gene, pork (*Sus scrofa*)

**DESAIN DAN STUDI PERFORMA METODE PROBE TaqMan  
REAL-TIME POLYMERASE CHAIN REACTION  
SPESIFIK TERHADAP GEN ATPase 6 BABI (*Sus scrofa*)**

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**INTISARI**

Telah dilakukan metode *real-time polymerase chain reaction* menggunakan probe TaqMan spesifik terhadap urutan gen ATPase 6 pada DNA mitokondria babi. Penelitian ini bertujuan untuk mendesain dan mempelajari spesifisitas dan kinerja dari primer dan probe TaqMan dalam mendeteksi babi sebagai metode deteksi spesies dalam daging.

Probe TaqMan dan primer didesain menggunakan software PrimerQuest dan uji homologinya dengan menggunakan BLAST. Uji kinerja dilakukan dengan menguji spesifisitas pada sampel DNA yang berasal dari daging babi, anjing, tikus, sapi, ayam, kambing, dan kuda. Uji presisi meliputi proses amplifikasi kontrol positif sebanyak 10 kali. Penentuan batas deteksi dengan mengamplifikasi templat DNA yang sudah diencerkan dari 50 ng menjadi 5 pg dan amplifikasi pada campuran templat DNA babi dan sapi dengan konsentrasi babi 1, 2, 5, 10, 25, 50, and 100% di dalam campuran tersebut.

Didapatkan primer forward (5'-CACACCCACCACACA ACTAT-3'), reverse (5'-GGTAGAAAGTGGGCTAGTGATG-3'), dan TaqMan probe dengan urutan (5'-FAM/TCAGCAACC/ZEN/GTATTCACAGGATTCCG/3IABkFQ/-3') sebagai hasil desain yang mempunyai target area di gen ATPase 6 pada babi. Probe dan primer mampu secara spesifik membedakan DNA babi terhadap spesies lainnya. Metode deteksi ini mempunyai presisi yang baik dan juga mampu mendeteksi DNA babi hingga 5 pg dan pada 1% kontaminasi babi dalam campuran.

Kata kunci: analisis daging, probe TaqMan, *real-time PCR*, gen ATPase 6, babi (*Sus scrofa*)