DESAIN DAN UJI KINERJA *PROBE* TaqMan *REAL-TIME POLYMERASE CHAIN REACTION* (RT-PCR) SPESIFIK ANJING (*Canis lupus familiaris*) DENGAN GEN SITOKROM-B DNA MITOKONDRIA

Efi Qurnia Dewi

13/347229/PA/15177

INTISARI

Telah dilakukan desain dan uji kinerja terhadap *real-time* PCR dengan *probe* TaqMan spesifik anjing (*Canis lupus familiaris*) dengan target gen sitokrom-b DNA mitokondria. Penelitian ini bertujuan mengembangkan metode uji spesifik untuk mendeteksi kontaminasi daging anjing berbasis DNA.

Penelitian ini dimulai dari mendesain *forward primer*, *reverse primer* dan *probe* TaqMan secara *in silico* berdasarkan DNA mitokondria *Canis lupus familiaris.* Hasil desain *primer* dan *probe* diuji spesifitasnya terhadap DNA anjing, tikus, babi, sapi, ayam, kambing dan kuda serta dilanjutkan uji presisi dengan pengulangan, penentuan batas deteksi yang berupa uji sensitivitas dengan pengenceran DNA anjing, dan uji kontaminasi dengan pencampuran antara DNA anjing dan DNA sapi.

Hasil desain yang diperoleh dari lima set terbaik dengan metode *in silico* yaitu *forward primer* 5'-TGGACAAAGCAACCCTAACA-3', *reverse primer* 5'-CCGGTTTCGTGTAGAAATAGGA-3', dan *probe* TaqMan 5'-/56-FAM/TCATCCTCC/ZEN/CTTTCATCGCAGC/3IABkFQ yang dapat mengamplifikasi secara spesifik terhadap DNA anjing (*Canis lupus familiaris*). Hasil uji kinerja yaitu *primer* dan *probe* TaqMan menunjukkan presisi yang baik dengan nilai standar deviasi relatif (RSD) sebesar 2,36% dan memenuhi parameter keberterimaan nilai RSD yaitu 25% serta dapat mendeteksi kontaminasi daging anjing hingga 5,00 pg dengan perkiraan jumlah kopi DNA cetakan sebesar 0,95 molekul/µL dan konsentrasi 1%.

Kata kunci : *Real-time Polymerase Chain Reaction*, sitokrom-b, *probe* TaqMan, anjing (*Canis lupus familiaris*)

DESIGN AND PERFORMANCE STUDY OF TaqMan *PROBE* REAL-TIME POLYMERASE CHAIN REACTION (RT-PCR) ASSAY SPECIFIC TO DOG (*Canis lupus familiaris*) BASED ON CYT-B GENE MITOCHONDRIAL DNA

Efi Qurnia Dewi

13/347229/PA/15177

ABSTRACT

Design and performance tests has been performed on TaqMan probe with real-time PCR specific to dog (*Canis lupus familiaris*) using the cytochrome-b mitochondrial DNA as gene target. This study aimed to develope the specific test method for detection of dog meat contamination based on DNA.

This experiment was started from designing forward primer, reverse primer and TaqMan probe using in silico method based on the mitochondrial DNA of dog (*Canis lupus familiaris*). Primers and probe design were tested for its specificity against the DNA of dog, rat, pig, cow, chicken, goat and horse then continued with precision tests by repetation method, determination of limit of detection as a sensitivity test by dog's DNA dilution method, and contamination test by mixing between the DNA of dog and cow.

The design results obtained from the best five sets with the in silico method are forward primer 5'-TGGACAAAGCAACCACAACA-3, reverse primer 5'-CCGGTTTCGTGTAGAAATAGGA-3 ', and TaqMan probe 5' - / 56-FAM / TCATCCTCC / ZEN / CTTTCATCGCAGC / 3IABkFQ that could amplify dog's DNA so that it is specific to the dog (*Canis lupus familiaris*). Performance test showed that primers and TaqMan probe has good precision with relative standard deviation (RSD) value of 2,36% and complied with the parameter of RSD value acceptability of 25% and could detect dog meat contamination up to 5.00 pg with estimated copy number of DNA template equal to 0,95 molecule/µL and concentration of 1%

Keywords: Real-time Polymerase Chain Reaction, cytochrome-b, TaqMan probe, dog (*Canis lupus familiaris*)