DNA FINGERPRINTING OF MARBLE GOBY, *Oxyeleotris marmoratus* USING RAPD-PCR TECHNIQUE

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By

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APPROVAL AND CERTIFICATION FORM

I certify that the report of this final year project entitled 'DNA Fingerprinting of Marble Goby, *Oxyeleotris marmoratus* Using RAPD-PCR Technique' by LEONG LOK PING, metric no. UK2532 have been read and all the alteration and correction recommended by Examiners have been done. This thesis submitted to Department of Biological Science, have been accepted as fulfilment of the requirement for degree of Bachelor Educational Science (Biology) in Faculty of Science and Technology, Kolej Universiti Sains Dan Teknologi Malaysia, Universiti Putra Malaysia.

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ABSTRACT

Marble goby (*Oxyeleotris marmoratus*) from the family Eleotridae is a commercially important fish species of the large suborder, Gobiodei. Random Amplified Polymorphic DNA based on Polymerase Chain Reaction (RAPD-PCR) technique was chosen in this study owing to its effectiveness that applied to a wide range of organisms. The aim of this study were to establish the DNA fingerprinting of *O. marmoratus*, to verify the genetic variability and to analyze the degree of polymorphism on DNA level marker of this species. Genomic DNA of 20 samples was extracted from the muscular tissues using Phenol-Chloroform Protocol technique. The purity of DNA samples ranged from 1.301-1.807, quantitatively estimated from the ratio between the reading of absorbence at 260nm and 280nm (OD_{260}/OD_{280}) in UV-Spectrophotometer. The purity of DNA also qualitatively observed through the appearance of single band formed on a 0.8% (w/v) agarose gel stained with 0.5µg/mL ethidium bromide. Quantity of DNA samples ranged from 270ng/µL - 685ng/µL. A total of 19 primers with 60-70% GC content were tested for their ability to amplify fragments from each DNA sample separately. The PCR products were then electrophoresed in 1.7% (w/v) agarose gel stained with 0.5µg/mL ethidium bromide. Three primers - OPA07, OPA09 and OPA20 were chosen for further study. The primers generated a total of 28 loci (fragments) with 91.7% polymorphic loci (25 fragments) and the RAPD banding ranged from 1-10 fragments with 350-1750bp. The similarity index among individuals was 0.442 ± 0.123 whereas genetic distance value was 0.400 ± 0.167. 550bp and 400bp were found to be diagnostic markers of *O. marmoratus*. Further studies on this species should be carried out in order to fulfill the needs of conservation and propagation of *O. marmoratus*.

ABSTRAK

Ikan Ketutu (*Oxyeleotris marmoratus*) yang berasal dari famili Eleotridae adalah spesis ikan dalam suborder Gobiodei yang mempunyai nilai kepentingan perdagangan. Pemilihan teknik Amplifikasi Rawak Polimorfik DNA yang berdasarkan Tindak Balas Rantai Polimerase (RAPD-PCR) dalam kajian ini disebabkan oleh keberkesanannya yang telah ditunjukkan pada banyak organisma. Tujuan kajian ini adalah untuk memaparkan pencapjarian DNA ikan ketutu, *O. marmoratus*, memeriksa kepelbagaian genetik serta menganalisa darjah polimorfik pada tahap penanda DNA spesis ini. DNA genomik dalam 20 contoh ikan diekstrak dari tisu otot dengan teknik Fenol-Kloroform. Ketulenan DNA ikan didapati berada dalam julat 1.301-1.807. Ia diperolehi secara kuantitatif dengan penganggaran nisbah antara bacaan penyerapan pada 260nm dan 280nm (OD_{260}/OD_{280}) dalam UV-Spektrofotometer. Ketulenan DNA diperhatikan melalui kewujudan jalur-jalur tunggal yang terbentuk pada 0.8% (w/v) gel agaros yang telah diwarnakan dengan 0.5µg/mL etidium bromida. Kuantiti DNA ikan berada dalam julat 270ng/µL-685ng/µL. Sembilan belas primer dengan kandungan GC sebanyak 60-70% diuji untuk kebolehan mereka mengamplifikasikan fragmen DNA ikan secara berasingan. Hasil PCR dilarikan melalui proses elektroforesis pada 1.7% (w/v) gel agaros yang diwarnakan dengan 0.5µg/mL etidium bromida. OPA07, OPA09 dan OPA20 telah dipilih untuk kajian seterusnya. Ketiga-tiga primer menghasilkan jumlah 28 loci dengan 91.7% (25 fragmen) daripada loci-loci tersebut adalah polimorfik. Jalur-jalur RAPD didapati berjulat antara 1-10 fragmen dengan 350-1750bp. Penunjuk Kesamaan (Similarity Index) antara individu adalah 0.442 ± 0.123, manakala nilai Jarak Genetik adalah 0.400 ± 0.167. Penanda Diagnostik untuk *O. marmoratus* yang diperolehi adalah 550bp dan 400bp. Kajian lanjut terhadap spesis ini perlu dijalankan untuk memenuhi keperluan pemuliharaan dan pembiakan *O. marmoratus*.