DNA FINGERPRINTING OF GREEN-LIPPED MUSSEL, *Perna viridis* USING RAPD-PCR TECHNIQUE

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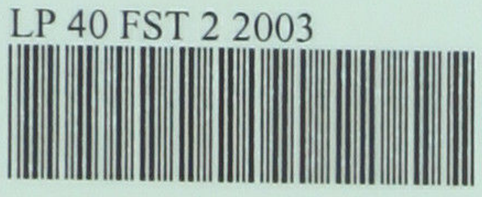

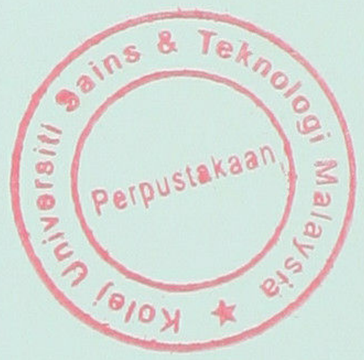

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By:

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This project report is submitted in partial Fulfillment of the requirements
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APPROVAL AND CERTIFICATION FORM

I certify that the report of this final year project entitled ' DNA Fingerprinting of Green-lipped Mussels, *Perna viridis* using RAPD-PCR Technique' by SHEN WAI SAN, matric no. UK4416 have been read and all the alteration and correction recommended by the examiners has been done. This thesis submitted to Department of Biological Sciences, have been accepted as fulfillment of requirement for degree of Sarjana Muda Science in Biological Science, Faculty Science and Technology, University College of Science and Technology Malaysia (KUSTEM).

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ABSTRAK

Perna viridis dari famili Mytilidae adalah spesis yang penting dari segi komersil dan sumber protein yang murah. Kajian ini tentang pencapjarian DNA kupang (*P. viridis*) menggunakan Teknik Amplifikasi Rawak Polimorfik DNA berdasarkan Tindak Balas Rantai Polimerase (RAPD-PCR) dari dua lokasi iaitu Pulau Ketam, Selangor dan Kampung Balak, Sungai Sengkawan, Port Dickson, Negeri Sembilan. Objektif kajian ini adalah untuk mengesahkan kepelbagaian genetik menggunakan (RAPD-PCR) dan menganalisis kadar polimorfik sampel dari dua lokasi yang berbeza. Teknik RAPD-PCR digunakan dalam kajian ini kerana keberkesannannya yang telah ditunjukkan dalam banyak organisma termasuk haiwan dan tumbuhan. Lima genomik DNA diekstrak dari setiap lokasi dari tisu "mantle-edge" menggunakan teknik protokol Fenol-Kloroform. Ketulenan sampel DNA adalah dari 1.57-2.38 diukur dengan menggunakan bacaan penyerapan pada 260 nm dan 280 nm dalam UV-Spektrofotometer. Kualiti ketulenan diperhatikan dengan kewujudan jalur-jalur tunggal terbentuk pada 0.8% (w/v) gel agaros yang telah diwarnakan dengan 0.5 μg/mL ethidium bromida. Kuantiti sample DNA adalah dari 275.0-1080.0 ng/μL. Dalam proses PCR, 20 primer dengan kandungan GC 60-70% diskrin ke atas salah satu sampel DNA untuk melihat kebolehan amplifikasi setiap primer. Selepas pengskrinan, tiga primer yang menjana jalur yang jelas - OPA07, OPA09 dan OPA20 di pilih untuk kajian seterusnya. Tiga primer itu menjana 59 loci dengan 86.06% (51 fragmen) polimorfik dan jalur RAPD berjulat 1-14 fragmen dengan 300-2460bp. Similariti indek antara individu adalah 0.24-0.81 manakala jarak genetik pula bernilai 0.19-0.57. Penanda Diagnostik untuk *P. viridis* yang di perolehi ialah 650 bp, 700 bp, 850 bp, 1000 bp dan 1650 bp. Kajian lebih lanjut pada spesis ini boleh dijalankan menggunakan jumlah individu dan primer yang lebih banyak supaya data yang lebih tepat dapat di perolehi dan polimorfik genetik *P. viridis* dapat lebih di ketahui.

ABSTRACT

Perna viridis from the family Mytilidae is a mussel species of commercial value for cheap protein source. A Study on DNA fingerprinting of Green-lipped mussel (*P. viridis*) using Random Amplified Polymorphic DNA based on Polymerase Chain Reaction (RAPD-PCR) technique from two locations, Pulau Ketam, Selangor and Kampung Balak, Sungai Sengkawan, Port Dickson, Negeri Sembilan was conducted. Objectives of this study are to verify genetic variability using RAPD-PCR technique, and to analyze the degree of polymorphism from two different locations of this species. RAPD-PCR technique was chosen because its effectiveness can be applied to wide range of organisms including plants and animals. Five genomic DNA samples were extracted from each location using the mantle-edge tissue with Phenol-Chloroform Protocol Technique. The purity of the DNA samples ranged from 1.57-2.38 measured by the reading of absorbance at 260 nm and 280 nm using UV-Spectrometer. The purity of DNA samples qualitatively obtained on 0.8% (w/v) agarose gel stained with 0.5 µg/mL of ethidium bromide was optimal. Quantities of DNA samples ranged from 275.0-1080.0 ng/µL. In PCR process, a total of 20 primers with 60-70% GC content were screened on one DNA sample to see their ability to amplify fragments from each different sample. After the screening procedure, three primers that generate clear band - OPA07, OPA09 and OPA20 were chosen for further study. The three primers generated 59 loci (fragments) with 86.06% polymorphic loci (51 fragments) and the RAPD banding ranged from 1-14 fragments with 300-2460 bp. The similarity index among individual was 0.24-0.81 while the genetic distance value was 0.19-0.57. 650 bp, 700 bp, 850 bp, 1000 bp and 1650 bp were found to be diagnostic markers of *P. viridis*. Further studies on this species with more samples and primers should be carried out to get a more convincing reading and in order to know more about *P. viridis* genetic polymorphism.