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DIFFERENT PRESERVATIVES AND DNA EXTRACTION METHODS FOR TISSUES OF *ANADARA OVALIS* (BIVALVE) IN PCR AMPLIFICATION STUDY

By

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JABATAN SAINS BIOLOGI
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PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: DIFFERENT PRESERVATIVES AND DNA EXTRACTION METHODS FOR TISSUES OF *ANADARA OVALIS* (BIVALVE) IN PCR AMPLIFICATION STUDY oleh Aisyah Syairah Binti Ab Rahman, no. matrik: UK8322 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah Sarjana Muda Sains (Sains Biologi), Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

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%	Percentage
^0C	Degree Celsius
l X	One Time
bp	Base pair
C	Cytosine
cm	Centimeter
dH_2O	Distilled water
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotides mixture
EDTA	Ethyylenediaminetetraceatic acid
TNES	Tris Hidrochloride, Sodium Chloride, EDTA, Sodium Deodocyl Sulphate (SDS)
g	Gram
G	Guanocine
M	Molarity
µg	Microgram
µl	Microlitre
µM	Micromolar
mg	Milligram
ml	Mililitre

mM	Milimolar
min	Minutes
ng	Nanogram
OD	Optical Density
PCR	Polymerase Chain Reaction
pM	Picomole
RAPD	Random Amplified Polymorphic DNA
rpm	Rotation per minute
sec	Second
TBE	Tris-borate-EDTA-buffer
TE	10Mm tris-Cl, 1mM EDTA
Tris-HCl	Tris [Hydroxymethyl] aminomethane hydrochloride
UV	Ultra violet
V	Volt
v/v	Volume/volume
VDS	Video Documentation System
w/v	Weight/volume

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ABSTRACT

Anadara ovalis also known as "kerang bulu" in Malay and blood ark in English is belongs to famili of Arcidae is a filter feeder. Two types of preservative were used to preserve the samples there were ethanol 95% and TNES urea buffer. The purity and quantity of DNA from different preservatives of *Anadara ovalis* were measured using UV Spectrophotometer and electrophoresis gel. The purity of tissue samples was gained using Phenol Chloroform Protocol and Wizard Genomic DNA Purification kit extraction. Both methods of DNA extraction were applied to determine the effective and best methods for DNA extraction of *Anadara ovalis.* The purity of blood ark samples were measured from the ratio between reading absorbance at 260nm and 280nm (OD_{260}/OD_{280}) using UV Spectrophotometer. The ratio for TNES urea buffer using Kit extraction was ranged from 1.1531 to 1.3011 and range of DNA quantity were at 282.5 to 887.5µg/ml. The ratio for ethanol 95% using Kit extraction was ranged from 1.1803 to 1.3490 and range of DNA quantity were at 337.5 to 1562.5 µg/ml. The ratio for TNES urea buffer using phenol extraction was ranged from 1.0482 to 1.2727 and range of DNA quantity were at 217.3 to 385.0µg/ml. The ratio for ethanol 95% using phenol extraction was ranged from 1.6060 to 1.7157 and range of DNA quantity were at 1325 to 1720 µg/ml. RAPD technique had been applied in this study to investigate the effect of preservation agents to DNA purity of samples. A total of 26 RAPD fragments yielded from six primers (OPA01, OPA04, OPA05, OPA06, OPA07 and OPA8) for screening of RAPD primer.

PENGAWETAN DAN KAEDAH PENGEKSTRAKAN DNA YANG BERBEZA UNTUK TISU-TISU *ANADARA OVALIS* DALAM KAJIAN AMPLIFIKASI PCR

ABSTRAK

Anadara ovalis, juga di kenali sebagai "kerang bulu" tergolong dari famili Arcidae merupakan sejenis kerangan pemakan hasil tapisan. Dua jenis larutan pengawet digunakan untuk mengawet sampel *Anadara ovalis* iaitu larutan penimbal Urea TNES and larutan etanol 95%. Larutan pengawet yang berlainan digunakan untuk mengkaji kesan bahan pengawet keatas ketulenan dan kuantiti DNA *Anadara ovalis*. Ini dijalankan untuk menentukan teknik pengawetan terbaik untuk sample *Anadara ovalis*. Ketulenan DNA diperolehi melalui dua kaedah pengekstrakan DNA iaitu Kaedah Fenol Kloroform dan Kit Wizard Genomik Purifikasi DNA (Promega). Kedua-dua jenis pengekstrakan dijalankan untuk menentukan kaedah yang terbaik bagi mengekstrak tisu DNA dari larutan pengawet yang berbeza. Ketulenan sampel ''kerang bulu'' diukur dari nisbah bacaan penyerapan pada 260nm dan 280nm ($OD_{260/280}$) menggunakan UV Spectrophotometer. Nisbah DNA adalah pada julat 1.0482 hingga 1.6096. Kuantiti DNA mempunyai julat antara 217.3 dan 1760µg/ml. Teknik RAPD telah diaplikasikan untuk mengkaji kesan pengawetan terhadap sampel DNA. Sejumlah 26 fragmen RAPD didapati dari enam primer (OPA 01, OPA 04, OPA 05, OPA 06, OPA 07, dan OPA 08).