





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

By

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Research Report submitted in partial fulfillment of  
The requirements for the degree of  
Bachelor of Science (Biological Sciences)

Department of Biological Sciences  
Faculty of Science and Technology  
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA  
2006

**1100046005**

This project should be cited as:

Aisyah Syairah, A.R. (2006) Different Preservatives and DNA Extraction Methods for Tissues of *Anadara ovalis* (Bivalve) in PCR Amplification Study. Undergraduate thesis, Bachelor of Science in Biological Science, Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia, Terengganu.

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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 memperoleh ijazah Sarjana Muda Sains (Sains Biologi), Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

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## ACKNOWLEDGEMENT

First at all, I would like to thank you to Allah Al-Mighty for His guidance and bless during my performing for this project all the year. Thank you to all individuals that involve directly or indirectly that have gave me moral support and encouragement including Biological Sciences Department staff and lecturers. Thank you to Kolej Universiti Sains dan Teknologi Malaysia which had gave me a lot of knowledge and experiences.

I would like to thanks my beloved parent, Dr Ab Rahman Abdullah and Mrs Nor'Aini Mohd Nor for being supportive and encouraging me to finish my study and complete my final project. Thankful to my adorable family members, thank you and I love you.

Therefore, I would like to express my gratitude to my supervisor and co-supervisor, Miss Wan Bayani Wan Omar and Dr Zaleha Kassim for their guide to design and perform this project. This project was successfully done with their knowledge, advices and expert in molecular technology.

Beside that, thank you to my project members, course mates and friends that always been supportive and willing to share knowledge in completing this project. Gratefulness to all my housemates, Fatmawati, Melati, Che Roslaily and Nurul Hazwani for their patience and understanding. Thank you.

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## LIST OF ABBREVIATION

%	Percentage
<sup>o</sup> C	Degree Celsius
1 X	One Time
bp	Base pair
C	Cytosine
cm	Centimeter
dH <sub>2</sub> O	Distilled water
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotides mixture
EDTA	Ethylenediaminetetraacetic acid
TNES	Tris Hydrochloride, 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 887.5 $\mu$ g/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  $\mu$ g/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 385.0 $\mu$ g/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  $\mu$ g/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 1760 $\mu$ g/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).