[illegible] TWO DIFFERENT [illegible]ETHANES AND [illegible] TISSUES OF

FAUNUS ATER [illegible] IN PCB [illegible] STUDY

[illegible]

FAKULTI SAINS DAN TEKNOLOGI

[illegible] SAINS DAN TEKNOLOGI MALAYSIA

2[illegible]3

The use two different preservatives and dna extraction methods for tissues of Faunus Ater (snail) in pcr amplification study / Mazlina Muhamed.

THE USE OF TWO DIFFERENT PRESERVATIVES AND DNA EXTRACTION METHODS FOR TISSUES OF *FAUNUS ATER* (SNAIL) IN PCR AMPLIFICATION STUDY.

By:

Mazlina Binti Muhamed

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.

This project should be cited as:

Mazlina, M. 2006. The Use of Two Different Preservatives and DNA Extraction Methods for Tissues of *Faunus ater* (Snail) In PCR Amplification Study. Undergraduate thesis, Bachelor of Science in Biological Sciences, Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia, Terengganu. 62 pp.

JABATAN SAINS BIOLOGI
FAKULTI SAINS DAN TEKNOLOGI
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA

PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: THE USE OF TWO DIFFERENT PRESEVATIVES AND DNA EXTRACTION METHODS FOR TISSUES OF *FAUNUS ATER* (SNAIL) IN PCR AMPLIFICATION STUDY oleh Mazlina Binti Muhamed, no. matrik: UK 8103 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.

Disahkan oleh:

.....

Penyelia Utama

Nama: WAN BAYANI WAN OMAR

Cop Rasmi: PENSYARAH
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
21030 Kuala Terengganu, Terengganu.

Tarikh: 30/4/2006

.....

Penyelia Kedua (jika ada)

Nama: *Dr. Zaleha Binti Kassim*

Cop Rasmi *Pensyarah*
Jabatan Sains Samudera
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
21030 Kuala Terengganu.

Tarikh: 30/4/2006

.....

Ketua Jabatan Sains Biologi

Nama: PROF. MADYA DR. NAKISAH BT. MAT AMIN

Cop Rasmi: *Ketua*
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
(KUSTEM)
21030 Kuala Terengganu.

Tarikh: 4/5/2006

ACKNOWLEDGEMENTS

Assalamualaikum w.b.t....

First of all, I would like to express my sincere appreciation to my supervisor, Cik Wan Bayani Binti Wan Omar and also to my co-supervisor, Dr. Zaleha Binti Kassim from Department of Biological Sciences and Marine Sciences, Faculty of Science and Technology for their guidance, advice, encouragement and their understanding. Without their cooperation, patience and full support, this project will not succeed.

I also wish to convey my appreciation to my parents and family for their moral support and understanding.

I also want to thanks to all my friends and my project partners, whose always willing to give their fully support and their willingness to help me during the process of completing this project. Thank you very much for your encouragement, caring understanding and patience. Thank you.

TABLE OF CONTENTS

		Page
ACKNOWLEDGEMENTS		ii
LIST OF TABLES		v
LIST OF FIGURES		vi
LIST OF ABBREVIATIONS		vii
LIST OF APPENDICES		ix
ABSRTACT		x
ABSTRAK		xi
CHAPTER 1 INTRODUCTION		1
CHAPTER 2 LITERATURE REVIEW		4
2.1	Taxonomy and Morphology	4
2.2	Habitat and Distribution	6
2.3	Feeding and Importance	7
2.4	Reproduction and Growth	8
2.5	Tissue Preservation	8
2.6	DNA Extraction	10
2.7	Molecular Marker	11
2.8	Polymerase Chain Reaction (PCR)	12
2.9	Random Amplification of Polymorphic DNA (RAPD)	14
2.10	Agarose Gel Electrophoresis	16

CHAPTER 3 METHODOLOGY 18

3.1 Sampling of *Faunus ater* 18

3.2 Tissue Preservation 18

3.3 DNA Extraction 19

3.3.1 Wizard Genomic DNA Purification Kit (Promega) 19

3.3.2 Phenol – chloroform Extraction 20

3.4 Analysis 21

3.4.1 Analysis of DNA Quality by Agarose Gel Electrophoresis 21

3.4.2 Measurement of DNA Purity and Quantity 21

3.4.3 DNA Amplification and Screening of RAPD Primer 22

CHAPTER 4 RESULTS 25

4.1 Purity and Quantity of DNA 25

4.2 Screening of RAPD primer 32

CHAPTER 5 DISCUSSION 35

5.1 Purity and Quantity of DNA 35

5.2 Screening for RAPD Primer 36

5.3 DNA Amplification of RAPD Selected Primer 36

CHAPTER 6 CONCLUSION 38

REFERRENCES 39

APPENDICES 44

CURRICULAR VITAE 48

LIST OF TABLES

Table		Page
3.1	Code, sequences, nucleotides length and G + C content of primers used in RAPD analysis.	23
4.1	DNA purity and quantity of *Faunus ater* using Wizard Genomic DNA Purification Kit for fresh tissues, TNES – Urea buffer and ethanol 95 %.	30
4.2	DNA purity and quantity of *Faunus ater* using Wizard Genomic DNA Purification Kit.	30
4.3	DNA purity and quantity of *Faunus ater* using Phenol – chloroform extraction	31

LIST OF FIGURES

Figure		Page
2.1	The classification of *Faunus ater*.	5
2.2	The morphology of *Faunus ater*.	6
4.1	Genomic DNA extracted by Wizard Genomic DNA Purification Kit for ethanol 95 %, TNES – Urea buffer, and fresh tissues.	26
4.2	Genomic DNA extracted by Wizard Genomic DNA Purification Kit extraction.	27
4.3	Genomic DNA extracted by Phenol – chloroform extraction.	28
4.4	RAPD banding pattern for screening primer of *Faunus ater* using OPA01 – OPA10 from Wizard Genomic DNA Purification Kit.	33
4.5	RAPD banding pattern for screening primer of *Faunus ater* using OPA01 – OPA10 from Phenol – chloroform extraction.	34

LIST OF ABBREVIATIONS

bp	Basepair.
dNTP	Deoxynucleotide triphosphate.
DNase	Deoxyribonuclease.
EDTA	Ethylenediaminetetra acetic acid.
kb	Kilobase.
PCR	Polymerase Chain Reaction.
RNase	Ribonuclease.
SDS	Sodium Dodecyl Sulfate.
TBE	Tris – borate – EDTA.
%	Percentage.
°C	Degree celcius.
1X	One time.
cm	Centimeter.
dH_2O	Distilled water.
DNA	Deoxyribonucleotide acid.
g	Gram.
M	Molarity.
µg	Microgram.
µL	Microliter.
µM	Micromolar.
mg	Miligram.
mL	Mililitre.

mM	Milimolar.
ng	Nanogram.
OD	Optical Density.
ppt	Part per trillion.
rpm	Rotation per minute.
TE	Tris – EDTA buffer.
Tris-HCl	Tris (Hydroxymethyl) aminomethane hydrochloride.
UV	Ultra Violet.
V	Volt.
VDS	Video Documentation System.
v/v	Volume/ volume.
w/v	Weight/ volume.
sec	Second.
TNES – Urea	Tris – NaCl, EDTA, and Sodium Dodecyl Sulfate.
DF	Dilution Factor.
RNA	Ribonucleotides Acid.
G + C	Guanine + Cytosine.
CTAB	Hexadecyltrimethyl Ammonium Bromide.
RFLP	Restriction Fragment Length Polymorphism.
AFLP	Amplified Fragment Length Polymorphism.
SNP	Single Nucleotide Polymorphism.
EST	Expressed Sequence Tag.
RAPD	Random Amplification of Polymorphic DNA.
DMSO – NaCl	Dimethyl Sulfoxide – Natrium Chloride.

LIST OF APPENDICES

Appendice		Page
Appendix A	List of present and absent band.	45
Appendix B	Apparatus used in this study	46

ABSTRACT

The main objectives of this study are to measure the purity and quantity of the DNA from ethanol 95 % and TNES – Urea buffer and to compare the efficiency of DNA extraction technique between Wizard Genomic DNA Purification Kit and Phenol – chloroform extraction. Ethanol 95 % and TNES – Urea buffer were used to preserved *Faunus ater* tissues for three month. Tissues from TNES – Urea buffer were extracted and obtained the genomic DNA as it shows a clear band compared to ethanol preservation that shows a degraded band when examined on 1.0 % agarose gel elctrophoresis. The ranged of DNA purity of *Faunus ater* from Phenol – chloroform extraction is higher than Wizard Genomic DNA Purification extraction when it measured using a UV Spectrophotometer by calculating the ratio of absorbance reading at 260 nm and 280 nm. The Phenol – chloroform extraction also produced higher DNA concentration compare to Wizard Genomic DNA Purification extraction ranged between 377.5 ng / µL to 855.0 ng / µL.

PENGGUNAAN DUA JENIS PENGAWET DAN KAEDAH PENGEKSTRAKAN DNA YANG BERBEZA DARIPADA TISU – TISU *FAUNUS ATER* (SIPUT) DALAM KAJIAN AMPLIFIKASI PCR

ABSTRAK

Tujuan penyelidikan ini adalah untuk mengukur ketulenan dan kuantiti DNA daripada tisu yang diawet didalam etanol 95 % dan TNES – Urea buffer dan juga untuk membezakan teknik yang terbaik untuk mengekstrak DNA diantara kaedah 'Wizard Genomic DNA Purification Kit" dan kaedah fenol – klorofom. Etanol 95 % dan TNES – Urea buffer digunakan untuk mengawet tisu *Faunus ater* selama tiga bulan. Sampel tisu yang diawet didalam TNES – Urea buffer digunakan untuk diekstrak bagi mendapatkan DNAnya. Hal ini kerana sampel tisu yang diawet didalam larutan tersebut menunjukkan jaluran DNA yang jelas jika dibandingkan dengan sampel tisu yang diawet didalam etanol 95 % yang menunjukkan jaluran DNA yang telah terdegradasi apabila diperiksa dengan menggunakan 1.0 % gel agaros elektroforesis. Julat ketulenan DNA bagi sampel *Faunus ater* yang diperolehi dengan menggunakan kaedah fenol – klorofom adalah tinggi jika dibandingkan dengan menggunakan kaedah 'Wizard Genomic DNA Purification Kit' apabila diperiksa menggunakan UV Spectrophotometer dengan mengira nisbah pada absorban 260 nm dan 280 nm. Malah kaedah fenol – klorofom menunjukkan kuantiti DNA yang tinggi jika dibandingkan dengan kaedah 'Wizard Genomic DNA Purification Kit'.