

THE EFFECT OF DIFFERENT POLYMERIZED AND NON
POLYMERIZED CROSSLINKERS ON
SUSPENSION POLYMERIZATION STUDY

MUHD IZANI BIN MOHAMAD

COLLEGE OF ENGINEERING,
UNIVERSITY SAINS MALAYSIA

2008

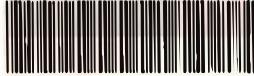
CM: 4733

1100046035

Perpustakaan
Universiti Malaysia Terengganu (UMT)



LP 34 FST 3 2006



1100046035

The use of two different preservatives and dna extraction
methods for tissues of Nerita sp (snail) in pcr amplification stuc
/ Mohd Izani Mohamed.

PERPUSTAKAAN
KOLEJ UNIVERSITI SAINS & TEKNOLOGI MALAYSIA
21030 KUALA TERENGGANU

1100046035		

Lihat sebelah

HAK MILIK
PERPUSTAKAAN KUSTEM

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

By

Mohd Izani Bin Mohamed

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:

Mohd Izani, M. 2006.. The Use of Two Different Preservatives and DNA Extractions Methods for Tissues of *Nerita sp* (bivalve or snail) in PCR Amplification Study. analysisUndergraduate thesis, Bachelor of Science in Biological Sciences, Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia, Terengganu. 37p.

No part of this project report may be produced by any mechanical, photographic, or electronic process, or in the form of phonographic recording, nor it may be stored in a retrieval system, transmitted or otherwise copied for public or private use without written permission from the author and the supervisor(s) of the project.



**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 USED OF TWO DIFFERENT PRESERVATIVES AND DNA EXTRACTION METHODS FOR TISSUES OF NERITA SP (SNAIL) IN PCR AMPLIFICATION STUDY oleh Mohd Izani Bin Mohamed no. matrik: UK 8041 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

WAN BAYANI WAN OMAR

Jabatan Sains Biologi

Fakulti Sains Dan Teknologi

Kolej Universiti Sains Dan Teknologi Malaysia

Mengabang Telipot,

21030 Kuala Terengganu,

Terengganu.

Nama:

Cop Rasmi:

Tarikh: 14/5/2006

Penyelia Kedua (jika ada)

Dr. Zaleha Binti Kassim,

Pensyarah

Jabatan Sains Samudera

Fakulti Sains dan Teknologi

Kolej Universiti Sains dan Teknologi Malaysia

21030 Kuala Terengganu

Nama:

Cop Rasmi

Tarikh: 14/5/2006

Ketua Jabatan Sains Biologi

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

Ketua

Cop Rasmi:

Tarikh: 14/5/2006

Jabatan Sains Biologi

Fakulti Sains dan Teknologi

Kolej Universiti Sains dan Teknologi Malaysia

(KUSTEM)

21030 Kuala Terengganu

ACKNOWLEDGEMENTS

Firstly, I would like to thank to the God because with His bless, I have finished my final project report. I also want to give my sincere appreciation to my supervisor, Cik Wan Bayani Wan Omar and also co-supervisor, Dr. Zaleha Kassim from Department of Biology Science and Marine Science, faculty of Science and Technology for their guidance, advice, encouragement and understanding.

Besides, I want to give my appreciation to my family for their support such as in materials, money and moral. Not forget to say a big thank to all my friends and my project partners who always give a support, caring, understanding and patience.

Lastly, I would like to thank again to all people who have involved in finishing this project. Without their cooperation and support, this project will not finish completely.

TABLES OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	v
LIST OF FIGURES	vi
LIST OF ABBREVIATIONS	vii
LIST OF APPENDICES	viii
ABSTRACT	ix
ABSTRAK	x
CHAPTER 1 INTRODUCTION	
1.1 Introduction	1
1.2 Objectives	3
CHAPTER 2 LITERATURE REVIEW	
2.1 Taxonomy and morphology.	4
2.2 Habitat and Distribution	7
2.3 Feeding	7
2.4 Reproduction and Growth	8
2.5 Polymerase Chain Reaction (PCR).	8
2.6 Random Amplified Polymorphic DNA (RAPD).	9
CHAPTER 3 METHODOLOGY	
3.1 Sampling of <i>Nerita sp</i>	11
3.2 Tissue Preservation	11

3.3	DNA Extraction	
3.3.1	Promega Wizard TM Genome DNA Purification Kit Extraction.	12
3.3.2	Phenol Chloroform Extraction	13
3.4	DNA Analysis	
3.4.1	Analysis of DNA Quality by Gel Electrophoresis	14
3.4.2	Measurement of DNA Purity and Quality	15
3.4.3	DNA Amplification and Screening of RAPD primer	15

CHAPTER 4 RESULTS

4.1	Tissue Preservation	18
4.2	DNA Extraction	18
4.3	Purity and Quality of DNA extraction.	20
4.4	Screening of RAPD PCR	22
4.5	Scoring Bands.	24

CHAPTER 5 DISCUSSION

5.1	Tissues Preservation	25
5.2	DNA Extraction	25
5.3	Purity and Quality of DNA extraction.	26
5.4	Screening of RAPD Primer	27

CHAPTER 6 CONCLUSION

REFERENCES	30
APPENDICES	34

LIST OF TABLES

Table		Page
3.1	Code, sequence, nucleotide length and G+C content of primers used in Random Amplified Polymorphic DNA (RAPD) analysis (Operon Technologies, Inc., Alabama, California)	16
4.1	DNA Purity and Quantity of <i>Nerita sp</i> samples in first month.	21
4.2	DNA Purity and Quantity of <i>Nerita sp</i> samples in second month.	21
4.3	DNA Purity and Quantity of <i>Nerita sp</i> samples in third month.	22
4.4	Scoring Band of RAPD fragments of screening TNES-Urea Buffer and Ethanol 95% from <i>Nerita sp</i> tissue.	24

LIST OF FIGURES

Figure		Page
1.1	The taxonomy of <i>Nerita sp.</i>	5
1.2	The examples of <i>Nerita sp</i> in ventral view.	6
1.3	The examples of <i>Nerita sp</i> in dorsal view.	6
4.1	Genomic DNA extracted by Wizard Genomic DNA Purification Kit (Promega) protocol.	19
4.2	Genomic DNA extracted by Phenol-Chloroform Method.	20
4.3	RAPD banding patterns for screening of TNES-Urea Buffer.	23
4.4	RAPD banding patterns for screening of Ethanol 95% Buffer	24

LIST OF ABBREVIATIONS

abs	-	absorbance
gm	-	gram
M	-	molar
mmol	-	milimol
nm	-	nanometer
U	-	unit
%	-	Percentage
°C	-	Degree Celsius
1X	-	One Time
A	-	Adenosine
bp	-	Base pair
C	-	Cytosine
cm	-	Centimeter
dH ₂ O	-	Distilled water
DNA	-	Deoxyribonucleic acid
dNTP mix	-	Deoxyribonucleotides mixture
EDTA	-	Ethylenediaminetetraacetic acid
g	-	Gram
G	-	Guanocine
M	-	Molarity
μg	-	Microgram
TNES	-	Tris NaCl EDTA-2Na SDS

LIST OF APPENDICES

Appendix	page
1.1 Apparatus for DNA extraction	
PCR machine – Eppendorf Thermal Cyclers	33
PCR machine – Eppendorf Thermal Cyclers	33
UV Transluminator	34
Centrifuge machine	34
Image Master VDS	35
Apparatus for electrophoresis	35

ABSTRACT

In this study, the objectives are to measure a quality and quantity of DNA from different preservatives and to determine the best technique to extract DNA from *Nerita sp.* From this study, the result showed the TNES-Urea Buffer is the better preservative than Ethanol 95% because all samples in this preservation get the clear bands of DNA in Genomic Extraction. DNA extraction from the Phenol-Chloroform method is the best extraction because all the samples showed a clear and sharpness banding of DNA but in the Kit Wizard Genomic DNA Purification Kit (Promega), the banding of Ethanol second month and third month showed degraded and not clear bands. In purity and quantity DNA, most of the *Nerita sp* DNA samples had an A260/280 ratio below 1.8 but only two Ethanol samples in second and third month on ratio above 2. In Screening RAPD primer, all the screening bands of the TNES-Urea Buffer and Ethanol 95% are clear and sharpness bands average between 1 to 8 fragments.

**PENGGUNAAN DUA JENIS PENGAWET DAN KAEDAH
PENGEKSTRAKKAN DNA YANG BERBEZA DARIPADA TISU-TISU
NERITA SP DALAM KAJIAN AMPLIFIKASI PCR**

ABSTRAK

Objektif utama kajian ini dibuat adalah untuk mengira kualiti dan kuantiti DNA daripada pengawetan yang berbeza dan untuk menentukan teknik yang terbaik dalam mengekstrakan DNA *Nerita sp.* Daripada kajian yang dibuat, didapati pengawet TNES-Urea Buffer lebih baik daripada Etanol 95% kerana semua sample dalam pengawetan ini mempunyai jaluran DNA yang jelas semasa melakukan proses pengekstrakan Genomic DNA. Pengekstrakan kaedah Phenol-Chloroform pula adalah pengekstrakan terbaik kerana jaluran DNA untuk semua jenis sampel yang terhasil adalah jelas. Bagi kaedah Genomic DNA Purification (Promega) Kit pula, hanya dua sampel yang menghasilkan jaluran DNA yang tidak jelas dan rosak. Dalam kualiti dan kuantiti DNA, semua sampel DNA *Nerita sp* mempunyai nisbah A260/280 di bawah 1.8 tetapi hanya dua sampel Ethanol bulan kedua dan ketiga mempunyai nilai atas daripada 2.0. Dalam RAPD primer Screening, jaluran DNA semua sampel bagi TNES-Urea Buffer and Ethanol 95% adalah jelas antara 1 hingga 8 fragmen.