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BIOLOGY II

Laboratory Manual Semester 2

SECOND EDITION

Edited by:
Mohd Ridwan Abd Rahman
Muhamad Ikhwan Idris
Rohaiza Daud

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TABLE OF CONTENTS

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PREFACE

This second edition of *Biology Laboratory Manual Semester II* is published to meet the needs of the students taking Biology course in Pusat Pengajian Pra Universiti (PPPU), Universiti Malaysia Sarawak (UNIMAS). The purpose of this manual is to provide the students with an organised, user-friendly manual to aid their understanding on the practical aspects of biology as well as enhancing learning and understanding of the lectures. This manual has been published based on reviews from PPPU Biology lecturers and advice from the experts consisted of experienced lecturers teaching in degree courses in UNIMAS.

Each section of the manual has been designed according to the standard of a scientific report format. In **Introduction**, brief explanation is provided to help the students form better idea on the background of the laboratory before they can start with the practical. This is followed by **Objectives**, which states the aim of the laboratory and what should be achieved at the end of the laboratory session. In **Materials**, a list of materials including the instruments and apparatus used for each laboratory is prepared for the student's reference. In **Methodology**, step-by-step procedure is explained in detail to guide the students in carrying out the practical correctly. The **Results** section is where the students record important observations and findings that they have obtained. **Discussions** section is for the students to explain the results that they have obtained and to relate with their learning units, before summarizing it in the **Conclusions** section.

This manual is also loaded with a number of additional features. This includes the tear-off section to enable the students to submit their report at the end of laboratory session. Besides that, the topics of the manual have been arranged according to their weekly learning units in their syllabus. Notes and theories/operating principles are also included as students' guidance. Relevant diagrams are inserted to enhance the understanding of the description in the manual. Post-Lab Questions are also prepared not only to validate the student's understanding but also to enhance their understanding of the laboratory procedure and recognise its applications.

Therefore it is hoped that the publication of this *Biology Laboratory Manual Semester II* will serve as a useful guide for the students in performing their laboratory sessions.

ABBREVIATIONS

%	percentage
°C	celsius
µl	microlitre
µS/cm	Microsiemens per centimetre
µmhos/cm	Micromhos per centimetre
AN	Ammoniacal nitrogen
BOD	Biochemical oxygen demand
CIA	Chloroform-isoamyl alcohol
cm	Centimetre
COD	Chemical oxygen demand
CTAB	Cetyltrimetylammonium bromide
Cyt c	Cytochrome c
ddH2O	Deionised distilled water
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DO	Dissolved oxygen
DOE	Department of Environment
eg	'exempli gratia' / for example
etc.	Et cetera
FNU	Fromazin nephelometric unit
FTU	Formazin turbidity Unit
GPS	Global Positioning System
i.e	'id est' / that is
KCl	Potassium chloride
mg/L @ mg/l	Milligram per litre
$MgCl_2$	Magnesium chloride
ml	Mililitre
mM	Milimolar

NaCl	Sodium chloride
NH_3N	Ammoniacal nitrogen
NTU	Nephelometric turbidity units
NWQS	National Water Quality Standards
PCR	Polymerase Chain Reaction
pH	Potential hydrogen
PPM	Parts-per-thousands
PPT	Parts-per-million
PSU	Practical salinity unit
rpm	revolutions per minute
sp.	Species
SS	Suspended solid
Taq	*Thermus aquaticus*
Tris – HCL	Tris(hydroxymethyl)aminomethane hydrochloride
UV	Ultraviolet
WQI	Water Quality Index

LABORATORY REGULATIONS

STUDENTS ARE STRICTLTY PROHIBITED TO ENTER LABORATORY WITHOUT PERMISSION OF THE AUTHORIZED PERSONNELS (LECTURERS, TUTORS AND LAB DEMONSTRATORS)

1. Attendance to the laboratory is compulsory. A Medical Certificate is needed for students who are unable to attend practical classes due to health reasons.
2. Students must be punctual to their practical sessions. Any latecomers will not be permitted to enter the laboratory.
3. Students must wear laboratory coat and proper/suitable shoes at all times. Sandals or open-toed shoes are strictly prohibited in the laboratory.
4. Read and follow all directions or methods of the experiment. Students should be prepared and understood all the procedures before performing the practical. Ask the lab demonstrators if there are any doubts about any part of the experiment.
5. Students are not allowed to eat and drink in the laboratory.
6. Students must wear safety goggles when working with chemicals, burners, or any substance that might get into the eyes.
7. Students must wear gloves whenever handling dangerous and corrosive chemicals.
8. Keep laboratory area clean and tidy. All instruments must be kept neat and clean at all times. DO NOT handle any equipment unless it is stated in the laboratory procedure.
9. Be serious and alert when working in the laboratory. Never mix chemicals just for fun because it might produce dangerous and explosive substances.
10. Do not waste any chemicals and take extreme care not to spill any material in the laboratory. Ask lab demonstrator about proper clean up procedure. Do not simply pour chemicals into the sink or trash container.
11. All accidents in the laboratory have to be reported to the lab demonstrators.
12. Take extreme care when handling scalpels or razor blades. Never point anything sharps towards other students. Students must immediately notify lab demonstrator if any student accidentally cut themselves or receive a cut.

13. Treat all living things with care and respect. Do not simply touch any organisms and specimens in the laboratory unless students have been given permission to do so. There are plants which are poisonous or with thorns and animals that may bite or scratched if alarmed.

14. Animals should be handled only if necessary. Lab demonstrator will give instructions on how to handle each species that may be brought into the laboratory.

15. Rinse any acids off the affected skin or clothing with water. Immediately inform lab demonstrator of any spillage.

16. Be aware of the location and proper use of safety equipment such as first aid-kit, fire extinguisher and emergency shower.

17. Students must clean and tidy the benches and return all equipment to its proper place when the experiment is completed. Wash hands after each experiment.

STUDENTS WHO FAIL TO ABIDE BY THE ABOVE STATED RULES AND REGULATIONS ARE SUBJECTED TO STRICT DISCIPLINARY ACTION

Biology Coordinator,
Pusat Pengajian Pra-Universiti,
Universiti Malaysia Sarawak.

LABORATORY INSTRUCTIONS

1. Students are advised to read the laboratory manual and make notes before attending each practical class in order to be adequately prepared. This will save the time and would ensure the students will be able to comprehend the practical in time.

2. Students are advised to bring the following items to every practical classes:
   i. Laboratory Manual
   ii. Any essential stationeries
   iii. Blank and Ruled A4 papers

3. Read and understand the practical guide in the laboratory manual before undertaking any practical exercise or experiment. Consult with the lecturers, demonstrators or lab assistant for any inquiry.

4. Submit a lab report to the lecturers/demonstrators at the end of each practical class in order to be marked.

5. The lab report depends on the nature of the practical: either experimental observations or drawings in plant or animal diversity topics.

6. Answer all questions and draw the observed specimens/slides/diagrams in the spaces provided.

7. Drawings or diagrams should be large (as a minimum of half a page), accurate, clear, titled and labeled accordingly. Scientific names of organisms should be written properly.

8. For drawings of specimens/slides observed under a microscope, the size of the objective must be given (X10, X40, etc.). Draw only what you visualized. YOU MUST NOT copy any drawings directly from other sources such as books or photocopied materials but students may use them as a guide.

SCIENTIFIC REPORT

* If the practical class involves experiments, students have to write a complete scientific report in the following format;:

1. Title
2. Introduction
3. Materials and Methods
4. Results
5. Discussion
6. Conclusion

Report should be in full sentences, past tense and comprehensible with precision, clarity and concise.

Title

The title must be short and precise.

Introduction

The introduction will be explaining the objectives of the experiment (maximum 100 words).

Materials and Methods

Summarize the procedures and the materials that were used.

Results

The data must be presented in tables, graphs or histograms whichever related, and with brief description.

Discussion

In the discussion, the results should be interpreted with valid explanation. In the case of obtained results are not as expected, students should discuss both the obtained and the expected results. Students may also make comparison on different methods and discuss on the related difficulties encountered.

Conclusion

The conclusion must be short and precise, based on the results and discussion

Practical 1
DNA Extraction Using Cetyltrimetylammonium bromide (CTAB)

Roberta Chaya Tawie Tingga and Mohd Ridwan Abd Rahman

Introduction

Deoxyribonucleic acid (DNA) isolation is an extraction process of DNA present in the nucleus of the cell. Methods used to isolate DNA are dependent on the source, age, and size of the sample. Isolation of DNA is needed for genetic analysis, which is used for scientific, medical, or forensic purposes. Common sources for DNA isolation include blood, hair, sperm, bones, nails, tissues, blood stains, saliva, epithelial cells, urine, bacteria, animal tissues, or plants. The isolation of DNA usually begins with lysis, or breakdown of tissue or cells. This process is essential for the destruction of protein structures and allows for release of nucleic acids from the nucleus. Lysis is carried out in a salt solution, containing detergents to denature proteins or proteases. In this experiment, the salt solution used is Cetyltrimetylammonium bromide (CTAB).

Objectives

1. To understand the process and principle of standard DNA isolation.
2. To extract DNA of animal tissue using Cetyltrimetylammonium bromide (CTAB) protocol.

Materials

1. Animal tissue
2. Micropipettes
3. Microcentrifuge tubes
4. Dissecting kit
5. Aluminum foil
6. Water bath
7. Centrifuge machine
8. CTAB solution

9. Proteinase K
10. Chloro-isoamyl alcohol (CIA)
11. Absolute ethanol (99.9% EtOH)
12. 70% ethanol (70% EtOH)
13. Sodium chloride (3M NaCl)
14. Deionized distilled water (ddh_20)

Methods

1. Cut up and mince a small amount of animal tissue on the aluminum foil.
2. Then put 700 µl of CTAB into 1.5 ml microcentrifuge tube.
3. Add 5 µl of Proteinase K and incubate in water bath at 65°C for 20-30 minutes or longer until the tissue is completely dissolved.
4. After tissue has dissolved, add 700 µl of CIA and inverse for 1 minute. Centrifuge at 13000 rpm for 10 minutes.
5. Take 550 µl of the upper layer of the supernatant and transfer to a newly labeled tube (do not touch the middle layer).
6. Add 550 µl of cold absolute EtOH. Then, spin the tube at 13000 rpm for 10 minutes.
7. Discard EtOH by pouring off. Make sure that the pellet is still intact at the bottom of the tube. Observe if the pellet is visible (yellow or white coloured pellet).
8. Add 550 µl of cold 70% EtOH, and 25 µl of 3M NaCl. Spin at 13000 rpm for 10 minutes.
9. Discard EtOH. Observe if DNA pellet is still in the tube.
10. Re-dissolve the pellet in 25-30 µl of (ddH_2O).
11. Run 1-2 µl of extraction product through 1% agarose gel electrophoresis.
12. Visualized the gel under UV transilluminator.

Name:_____ Matric No:_____ Group:___

Laboratory Report

Write **TWO (2)** pages of handwritten report based on the DNA extraction experiment that you have conducted today. By using your own words, this report must include a brief **Introduction** (2 marks), **Objectives** (2 marks), **Methodology** (5 marks), Results (6 marks – including gel documentation), **Discussion** (10 marks), **Conclusion** (2 marks) and list of **References** used (3 marks). **[30 marks]**

Name:_____ Matric No:_____ Group:___

Name: _____ Matric No: _____ Group: ___

Gel documentation **[3 marks]**

References **[3 marks]**

Name:_____ Matric No:_____ Group:___

Post Laboratory Questions

1. What is the role of CTAB solution in DNA isolation? **[3 marks]**

2. What does the Proteinase K do? **[1 mark]**

3. What does the CIA do and why? **[4 marks]**

Name: _____ Matric No: _____ Group: ___

4. How can we shorten the incubation time during the lysis process? **[3 marks]**

5. What is the use of every centrifugal step in the protocol? **[1 mark]**

6. Why does the DNA clumps together? **[4 marks]**

Practical 2
DNA Amplification Using Polymerase Chain Reaction (PCR)

Mohd Ridwan Abd Rahman and Roberta Chaya Tawie Tingga

Introduction

The polymerase chain reaction (PCR) is a technique to amplify the small amount of DNA up to a millions of copies by using the thermocycler. It involves enzymatic synthesis of the specific DNA including a combination between a DNA sample with the oligonucleotide primers, deoxynucleotide triphosphate (dNTPs) and the DNA polymerase in the buffer. There are three events that must take place in the PCR process; 1) template denaturation which is the most important process to make sure the cells' membrane are being lyses; 2) annealing of the primers and lastly; 3) the extension of the annealed primers using DNA Taq polymerase. The PCR technique is widely used in molecular biology, microbiology, genetics, diagnostics, clinical laboratories, forensic science, environmental science, hereditary studies, paternity testing, and many other applications.

Objectives

1. To explain the function of each component used in the PCR amplification.
2. To explain the process involved in the amplification of DNA.
3. To give hands-on experience to the students on DNA amplification using the thermocycler.

NOTES

- Precautions should be taken to guard against contamination of the reaction with trace amounts of DNA that could serve as templates.
- Disposable gloves should be worn throughout the preparation of the PCR.
- Always include a control that contains all the components of the PCR except the template DNA.

Name:_____ Matric No:_____ Group:___

Materials

1. 10x reaction buffer
2. $MgCl_2$ (25 mM)
3. dNTPs mix (10mM)
4. Primers : _____ gene;
   a. Forward - _____
   b. Reverse - _____
5. Template DNA
6. Taq DNA polymerase (5 u/µl)
7. Deionized distilled water (ddH_20)
8. Thermocycler

Methods

1. First, make a master mix in the 1.5 ml microcentrifuge tube. Add each components in the following order:

Table 2.1: Components of PCR master mix.

No.	Component	v	*x
		1x Reaction (25 µl)	y reactions
1	10x reaction buffer	2.5	
2	$MgCl_2$ (25 mM)	1.5	
3	dNTPs mix (10 mM)	0.5	
4	Forward (10 mM)	1.0	
5	Reverse (10 mM)	1.0	
6	ddH_2O	**z	
	Template DNA	*a*	
	Taq polymerase, (5 u/µl)	0.2	
	Total	25	

*x = (v) (y)

** z = 25 µl - ∑ reaction components

a = vary, depending on the quality and quantity of DNA Template

b = ∑ Component reaction 1 until 6

Name:_____ Matric No:_____ Group:___

2. Mix the components briefly. Spin down the mixture by pulsing in a microcentrifuge to bring all reaction components to the bottom of the tube.

3. Aliquot b μL into each 0.2 ml PCR tube.

4. Add a μL of template DNA to each reaction except for the negative control tube (DNA will be replaced by 1.5 μL of ddH_2O for the negative control).

5. Lastly, add 0.2 μL of Taq polymerase into each of 0.2 ml PCR tube.

6. Carry out amplification in the thermocycler with the following PCR parameter.

Table 2.2: PCR parameter

Step	Temperature (°C)	Time	No. cycles
Pre-Denaturation	95	_____	1
Denaturation	95	_____	} 30
Annealing	_____	_____	
Extension	72	_____	
Final Extension	72	_____	1
Soak	4	∞	∞

7. After amplification of the products, visualize PCR products using 2% agarose gel electrophoresis.

NOTES

- Make sure you collect the amplified products directly after the process reaches the soaking step.
- Never let the products soaked for more than half an hour since this action might cause damage to the thermocycler.

Name:_____ Matric No:_____ Group:___

Results **[5 marks]**