BIOLOGY II

Laboratory Manual

THIRD EDITION

Edited by
Mohamad Fhaizal Mohamad Bukhori
Maybelline Goh Boon Ling
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Universiti Malaysia Sarawak

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Preface

This third edition of Biology Laboratory Manual Semester 1 has been published to meet the needs of students taking Biology course in Pusat Pengajian Pra Universiti (PPPU), Universiti Malaysia Sarawak (UNIMAS). The purpose of the manual is to provide the students with an organised, interface to enable them to understand laboratory aspects of biology as well as making learning and understanding the lectures better. The manual has been published based on reviews from PPPU Biology lecturers and advice from experts comprising of experienced lecturers teaching in degree courses in UNIMAS.

Each section of the manual has been designed according to the standard of scientific report formatting. In **Introduction**, a brief overview of the practical is explained to help the students have better idea and understanding of the practical before they start. Then, followed by **Objectives**, which state the aim of the practical and should be achieved at the end of the session. In **Materials**, a list of materials including the instruments and apparatus used for each practical is prepared for the reference. In **Methodology**, step-by-step procedure is explained in detail to guide students in carrying out the practical correctly. **Results** section is where the students record important results and findings that they have obtained. **Discussions** section is for the students to explain the results that they have obtained to relate with their learning units, before concluding it in **Conclusions** section.

The manual is also filled with several additional features. One of it is the tear-off section to enable the students to submit the report at the end of laboratory session easily. Besides that, the topics of the practical have been arranged according to the weekly learning units in the academic session. Notes are also included for the student's guidance. Relevant figures, diagrams, and tables are incorporated to enhance the understanding of the description in the manual. Post-Laboratory Questions are also prepared not only to measure the student's understanding but also to enhance their understanding of the practical procedure and recognise its applications.

Therefore, it is hoped that the publication of this PRH 1026 Biology II Laboratory Manual will serve as a meaningful guide for the students in performing their laboratory sessions.

Acknowledgement

Special thanks and appreciation to all members of the Biology Course in Pusat Pengajian Pra-Universiti (PPPU), Universiti Malaysia Sarawak (UNIMAS), Mohamad Fhaizal Mohamad Bukhori, Maybelline Goh Boon Ling, Rohaiza Daud, Christharina S Gintoron, Roberta Chaya Tawie Tingga, Mohamad Razif Othman, Muhamad Ikhwan Idris, Mohd Aminudin Mustapha, Norfarahin Norwen, and Dr Mohd Ridwan Abd Rahman, who had been tirelessly involved in initiating, editing and compiling this laboratory manuals in order to make the third publication of this manual book a reality.

We also like to thank Tarmizi Mohammad Shukri for contributing the book design and photography for this PRH1026 Biology II Laboratory Manual.

STUDENTS ARE STRICTLTY PROHIBITED TO ENTER LABORATORY WITHOUT PERMISSION FROM THE AUTHORITY (LECTURERS, LABORATORY DEMONSTRATORS, LABORATORY ASSISTANTS)

1. Attendance to the laboratory is compulsory. An official and recognized Medical Certificate is needed for students who are unable to attend classes due to health reasons.

2. Students must be punctual to their practical class. Any latecomers will not be permitted to enter laboratory.

3. Students must always wear laboratory coat and shoes. Sandals or open-toed shoes are strictly prohibited in the laboratory.

4. Read and follow all directions or methods of the experiment/practical exactly as they are written. Students should be prepared and under stood all the procedures before the practical class. Ask the laboratory demonstrator if there are any doubts about any part of the experiment/ practical.

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 the laboratory area clean and tidy. All the instruments must be always kept neat and clean. Never handle any equipment unless it is stated in the experiment/practical procedure.

9. Be serious and alert when working in the laboratory. Never mix chemicals just for fun because they 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 the laboratory demonstrator about the proper clean-up procedure. Do not simply pour chemicals onto the skin or in the trash container.

11. All accidents in the laboratory must be reported to the lecturer, laboratory demonstrator, or laboratory assistant.

12. Take extreme care when handling scalpels or razor blades. Never point anything sharps towards other students. Students must immediately notify the laboratory 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 that are poisonous or have thorns and animals may bite or scratched if alarmed.

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

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

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

17. Students must clean their workbench and return all equipment to its proper place when the experiment/practical is completed. Wash hands after each experiment/practical.

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

Biology Course 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 prepare what to do in advance. This will save time and ensure that the students will comprehend the practical in time.

2. Students are advised to bring the following items before attending each class:

    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 experiment/practical. Consult with the lecturers, laboratory demonstrators, or laboratory assistants for any inquiries.

4. Submit the practical report to the lecturers or laboratory demonstrators at the end of each practical class to be marked if instructed.

5. The practical report depends on the nature of the practical: either full experiment or drawings in case of any topics.

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

7. Drawings or diagrams should be large, accurate, clear, titled and labelled accordingly. Scientific names of organisms should be written properly.

8. In case of drawings of the specimens/slides observed under a microscope, the size of the objective must be given (X10, X40, etc.). Draw only what you visualize. Strictly do 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 full experiments, students are required to write a complete scientific report as followed:

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

Report should be written in full sentences, past tense and comprehensible with precision, clarity, and economy of words.

Title

The title must be short and precise.

Introduction

The introduction can be between one to three paragraphs in explaining why the experiment is done and what are the objectives of the experiment.

Materials and Methods

Summarize the procedures and the materials that were used.

Results

The data must be presented in figures, tables, or graphs if any with a brief description.

Discussion

In the discussion, the results should be interpreted with the significance explained. The results also must be validated in view of the purpose of the experiment. In case of flawed results obtained, discuss the results that expected as well as those obtained. Students may also contrast the methods or discuss the difficulties if any.

Conclusion

The conclusion must be short and precise derived from the result and discussion.

µL	microlitre
CIA	chloro-isoamyl alcohol
CTAB	cetyltrimetylammonium bromide
ddH_2O	deionised distilled water
DNA	deoxyribonucleic acid
dNTPs	deoxynucleotide triphosphate
EtOH	ethanol
G1	growth or gap 1
G2	growth or gap 2
L	Linnaeus
mL	millilitre
MgCl2	magnesium chloride
NaCl	sodium chloride
PCR	polymerase chain reaction
rpm	round per minute
S	synthesis of DNA
sp	species
UV	ultraviolet

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

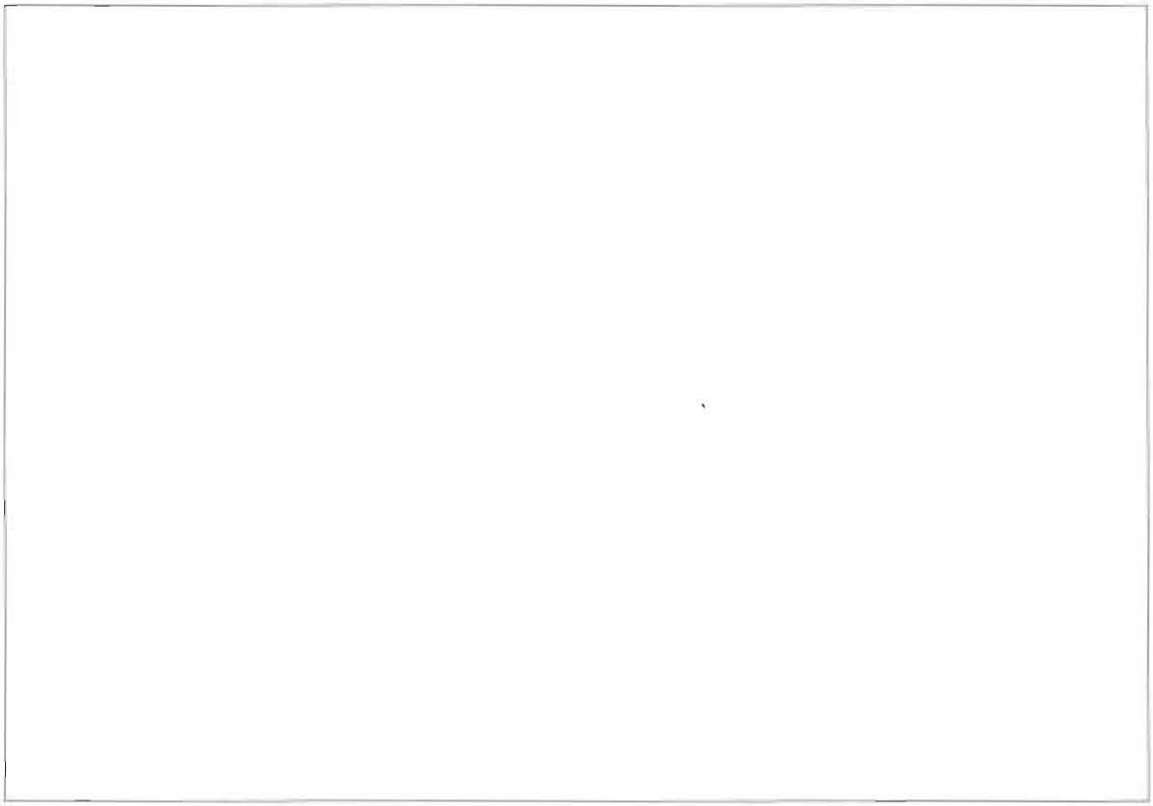
1. Animal tissue
2. CTAB solution
3. Proteinase K
4. Chloro-isoamyl alcohol (CIA)
5. Absolute ethanol (99.9% EtOH)
6. 70% ethanol (70% EtOH)
7. Sodium chloride (3M NaCl)
8. Deionised distilled water (ddH_2O)
9. Micropipettes
10. Microcentrifuge tubes
11. Dissecting kit
12. Aluminum foil

13. Disposable latex gloves
14. Water bath
15. Centrifuge machine

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 60°C for 20 to 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 to 30 µL of (ddH_2O).
11. Run 1 to 2 µL of extraction product through 1% agarose gel electrophoresis.
12. Visualized the gel under UV transilluminator.

Results [3 Marks]



[2 Marks]

Discussion [10 Marks

Conclusion [2 Marks]

Post Laboratory Questions

1. State the functions of CTAB solution in DNA isolation. **[3 Marks]**

2. State one functions of Proteinase K. **[1 Mark]**

3. State the functions of Chloro Isoamyl Alcohol (CIA). **[3 Marks]**

4. How to shorten the incubation time during the lysis process? **[2 Marks]**

5. State the functions of every centrifugal step in the protocol. **[1 Mark]**

6. What causes the DNA to clump together? **[3 Marks]**

Practical 2 DNA Amplification using Polymerase Chain Reaction (PCR)

Roberta Chaya Tawie Tingga, Mohd Ridwan Abd Rahman
and Mohd Aminudin Mustapha

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 is 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

A. Precautions should be taken to guard against contamination of the reaction with trace amounts of DNA that could serve as templates.

B. Disposable gloves should be worn throughout the preparation of the PCR.

C. Always include a control that contains all the components of the PCR except the template DNA.

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. Deionised distilled water (ddH_20)
8. Thermocycler

Methods

1. First, make a master mix in a 1.5 mL microcentrifuge tube. Add each of the component in the following order:

Table 2.1: Components of PCR master mix.

No.	Component	v	*x
		1x Reaction (25 µL)	**y reactions**
1	5x reaction buffer	5.0	
2	$MgCl_2$ (25 mM)	1.5	
3	dNTPs mix (10 mM)	0.5	
4	Forward (10 mM)	1.0	
5	Reverse (10 mM) ddH_2O	1.0	
6		**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

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	X	____	
Final Extension	72	____	1
Soak	72	____	
	4	____	∞

X – varies according to the gene and species used in the experiment

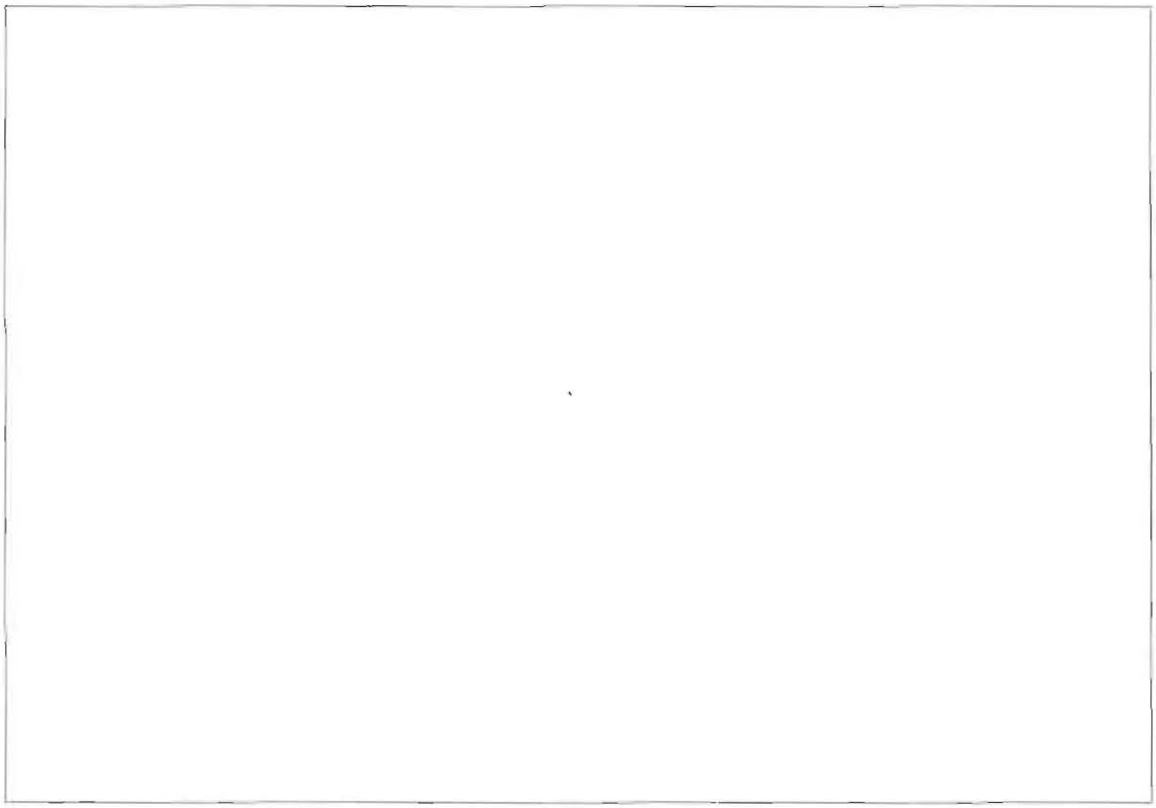
7. After amplification of the products, visualize PCR products using 2% agarose gel electrophoresis.

NOTES

A. Make sure you collect the amplified products directly after the process reaches the soaking step.

B. Never let the products soaked for more than half an hour since this action might cause damage to the thermocycler.

Results **[3 Marks]**



[3 Marks]

Discussion [10 Marks]

Conclusion **[2 Marks]**

Post Laboratory Questions

1. Briefly explain how denaturation of DNA occurs. **[2 Marks]**

2. State the functions of the reaction buffer in DNA amplification. **[2 Marks]**