MOLECULAR BIOLOGY

(PRINCIPLES AND PRACTICES)

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(Principles and Practices)

By

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Preface

Molecular Biology is a distinct discipline dealing with understanding the biological processes at molecular level. It is the result of coalescence of different disciplines like cell biology, genetics and biochemistry and has applications as divergent as plant and animal breeding, archaeology, diagnostic tests for a wide range of inherited conditions, diagnosis and treatment of chronic illnesses, desired manipulation of genome, decoding and exploiting the genetic information in the benefit of mankind, improving nutritional content of food crops and many more. In fact molecular biology techniques have played a decisive role in establishing modern biology as a qualitative and quantitative science. These techniques are based on a thorough understanding of principles of the biological processes as well as principles of modern instrumentation. Many-a-time students regard Molecular biology techniques merely as a collection of empirical instructions for performing qualitative and quantitative analysis. They follow instructions mechanically without understanding the theoretical considerations and principles that underlie various procedures. The aim of this book is to provide the readers an information capsule about some of the popular molecular biology techniques.

The book predominantly centers on a molecular biology laboratory which is routinely handled by undergraduates, post graduates as well as research students in the field of biotechnology. The first unit exclusively deals with the setting up of the laboratory, basic practices done routinely and safety guidelines about different biohazards. We hope this unit will be of immense help to the newcomers who are unaware of the dark side of the fascinating and so popular techniques of molecular biology. The second unit is focused on the various types of calculations involved while performing an experiment. This will guide the students how to prepare different solutions of required concentration and different buffers at different pH. The following units deal with the important techniques which are routinely carried out by students in their practical classroom as well as in their research assignments. Every technique is discussed in detail by giving a theoretical base in the starting and then discussing about principles, requirements as well as the methodology of the process. Some additional information which can make the experiment a success is given in every unit under the heading of 'Remember'. This additional information must always be remembered while performing these experiments.

In preparing this book the major emphasis has been given on clear and simple presentation in a language that is readily understandable by an average student. We are quite aware of the fact molecular biology is a very fast growing area and we really can not claim to include everything that the reader would look for. We have started looking forward to the possibility of modified versions by updating the content as well as incorporating the criticism and

suggestions from the scientific community who use this book. We owe every responsibility for any shortcomings.

In the end we like to acknowledge our family members for their support and patience. Dr Priyanka acknowledges the blessings and love of Nani ji, Mummy, Papa, Raj Dadi ji, and Ran Singh Dada ji. She also acknowledges the love and cheerful moments given by her brother Maj Amit, Sister-in-law Poonam and the support given by siseter-in-law Aruna. She can not forget to acknowledge her adorable kids Chaitanya and Chitrakshi whose presence has given fragrance to the life and made her family to blossom like a flower. In the end she acknowledges all the support, patience, guidance and above all the motivation given by her beloved husband Dr. Manoj. There were times when her morale was low enough to drop this assignment; it was only because of his constant motivation and love that she could lead towards completion of the book.

Dr Namita acknowledges all the love and affection given by her Papa, Mummy, Brother Dr. R. K. Singh, Sister, Mrs. Saraswati, Dr. Santosh, Dr. C.K. Singh, Seema, Brother In law Dr. K.C. banger, Dr. Jagjinder Singh and Dr. Birender laura.

We acknowledge the sincere efforts of Prof. R.K. Singh, for helping us in preparing the final design of the book as well as in selection of the right publisher for this manuscript. It was only because of his personal interest that book is published in such a short span.

In the end, we extend our sincere thanks to all our fellow educators and respected teachers (Prof. P.S. Bisen, Prof. V.K. Chowdhury, Prof R.K. Jain, Prof. D.P. Singh).

—AUTHORS

ABBREVIATIONS USED

AFLP	Amplified Fragment Length Polymorphism
CAPS	cleaved Amplified Polymorphic sequence
DarT	DNA qarray Technology
DNA	Deoxyribonuclic acid
EDTA	Ethylene diamine tetra-acetic acid
Kb	Kilobase
ISSR	Inter Simple sequence repeats
MAS	Marker Assistant selection
Min	Minute
MITEs	Miniature inverted reapeat transposable elements
PAGE	Polyacrylamide Gel electrophoresis
PCR	Polymerase chain Reaction
QTL	Quantitative Trait loci
rDNA	recombinant DNA
RAPD	Random Amplified polymorphic DNA
RFLP	Restriction Fragment length polymorphism
RNA	Ribonucleic acid
SNP	Single Nucleotide polymorphism
SSR	Simple Sequence Repeats
Tris	2-amino-2-hydroxymethylpropane-1,3-diol.

Unit I

MOLECULAR BIOLOGY LABORATORY— AN INTRODUCTION

Part 1 GENERAL LABORATORY FACILITIES

Contents

- **Basic Lay-out**
- **Personal Protective Equipment**
- **Research Equipments**

BASIC LAY-OUT

A standard molecular biology laboratory should be developed keeping in view the following points:

1. Lockable doors should be there for facilities that house restricted agents.
2. If developing a new laboratory, consider locating away from public areas.
3. Each laboratory should contain a sink for hand washing, Foot, knee, or automatically operated sinks are recommended.
4. Laboratory furniture must be capable of supporting anticipated loading and uses.
5. Spaces between benches, cabinets, and equipment should be accessible to easy cleaning.
6. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
7. Install biological safety cabinets in such a manner that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets to operate outside their parameters for containment. Locate biological safety cabinets away from doors,

from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment so as to maintain the required parameters.

8. All exit and entry laboratory doors should be equipped with Air curtain.
9. An eyewash station must be readily available.
10. Illumination should be adequate for all activities, avoiding reflections and glare that could impede vision.
11. Though there are no specific ventilation requirements, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
12. Proper Waste disposal facilities should be available for Bio hazard waste.
13. There should be adequate provision for following:
    (*a*) Gas, Water and electricity supplies
    (*b*) Distilled Water unit
    (*c*) A continuous supply of chemicals and glassware
    (*d*) Compressed air and vacuum lines
    (*e*) Pipette washer
    (*f*) Drying and draining racks
    (*g*) Storage space for chemicals, glassware, nutrient media
    (*h*) Water heater
    (*i*) Water bath
    (*j*) Acid proof baths for cleaning glassware
    (*k*) Automatic dishwasher
    (*l*) Glass automizer
    (*m*) Dispensing devices
    (*n*) Markers, labels and vita film
    (*o*) Micropipettes
    (*p*) Research equipments
    (*q*) Personal protective equipment

PERSONAL PROTECTIVE EQUIPMENT

Personal protective equipment, or PPE, is designed to protect researcher from injuries or illnesses resulting from contact with chemical, radiological, physical, electrical, mechanical, or other hazards. PPE includes a variety of devices and garments such as safety glasses, goggles, gloves, earplugs, coverwalls, and respirators. Using PPE is often essential, but it should not be used as a substitute for engineering, work practice, and/or administrative controls to prevent exposure to hazards. Engineering controls involve physically changing the work environment. An example of an engineering control would be a chemical fume hood. Administrative controls involve changing how or when lab workers do their jobs, such as scheduling work and rotating workers

to reduce exposures. Work practice controls involve training workers to perform tasks in ways that reduce their exposure to hazards. The personal protective equipments include:

(*a*) **Face and eye protection:** Safety glasses with side shields are required for work with hazardous chemicals. Ordinary prescription glasses are not adequate protection. Contact lenses can be worn safely if appropriate eye and face protection is also worn. Although safety glasses can provide satisfactory protection from injury from flying particles, they do not fit tightly against the face and offer little protection against splashes or sprays of chemicals. Splash goggles should be worn if there is a potential for splash in any operation involving chemicals. Full face shields with splash goggles should be worn when handling large quantities of chemicals, explosive, or highly hazardous chemicals. If work in the lab could involve exposure to lasers, ultraviolet light, infrared light, or intense visible light, specialized eye protection should be worn.

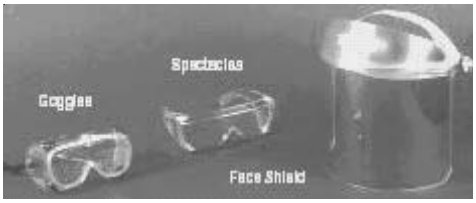

FIGURE 1.1

FIGURE 1.2

(*b*) **Hand protection:** Gloves appropriate to the hazard should be used. It is important that the hands and any skin that is likely to be exposed to hazardous chemicals receive special attention. Proper protective gloves should be worn when handling hazardous chemicals, toxic materials, materials of unknown toxicity, corrosive materials, rough or sharp-edged objects, and very hot or cold objects. Before the gloves are used, it is important that they be inspected for defects. The degradation and permeation characteristics of the glove material selected must be appropriate for protection from from the hazardous chemicals being handled. Glove selection guides (available from most glove manufacturers) should be consulted. Disposable latex gloves are very permeable to most chemicals. They are designed for use with biological hazards, and they should not be used for chemical protection. If latex gloves are used for biological hazards, be aware of latex allergy symptoms: skin rash, inflammation, respiratory irritation, and in rare cases, shock. Be sure to let your supervisor know if you have latex allergy. Gloves should be inspected before use, frequently during use, and replaced immediately if they are contaminated or torn. The use of double gloving may be appropriate in situations involving chemicals of high or multiple hazards. Hands should be washed after removing gloves. **Always remove gloves before leaving lab.**

FIGURE 1.3

(*c*) **Clothing:** Wear clothing that protects your skin. Wear long pants - no shorts or short skirts. Wear a lab coat for further protection. Lab coats should be worn with the front

fastened and the sleeves completely down, not rolled up. The coat sleeves keep splashes and aerosols from contacting your forearm and wrist. Have a plastic or rubber apron available for working with strong caustics or corrosives. Shoes should completely cover your feet; do not wear open toed shoes, sandals or—sleeper.

(*d*) **Respirator:** The primary method for the protection of laboratory personnel from airborne contaminants should be to minimize the amount of such materials entering the laboratory air. Engineering controls such as chemical hoods, biosafety cabinets, and local exhaust ventilation shall be used to contain and exhaust hazardous emissions. There should be very few instances in a laboratory when respiratory protection is necessary. Respirators may only be considered when engineering controls are not feasible or are inadequate to reduce exposures to acceptable levels.

(*e*) **Dust masks:** There are times when lab staff may wish to use respiratory protection, even when exposures are below the regulatory exposure limit, to provide an additional level of comfort and protection. For example, dust masks are often worn as a precaution when weighing toxic powders, even though there is no quantifiable exposure. However, if a respirator is used improperly **(dust masks are not appropriate for volatile chemicals)** or not kept clean, the respirator itself can become a hazard to the worker.

RESEARCH EQUIPMENT

Vacuum Pump

Bangalore Genie:

- Use for Gel/sample drying.
- Use with vacuum filtration unit.

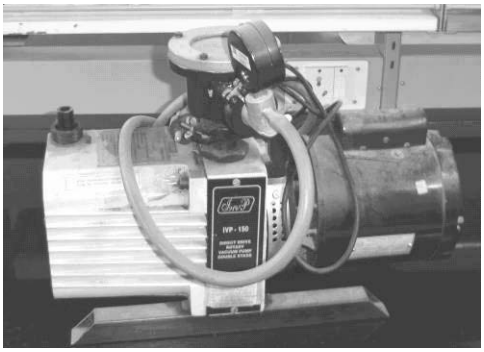

FIGURE 1.4

Inverted Microscope

Leica DMIL, Germany:

- Used For enumeration of cell.
- Inverted Microscope combined with a Visual Display Unit. Used for the observation of culture in tissue culture flasks and Petri-plates.
- Commonly used for counting the cell on haemocytometer.

FIGURE 1.5

Compound Light Microscope

ZEISS – 459306, NIKON® – ECLIPSE – E400:

- Compound Light microscope; have very high resolution power used for chromosomal observation on the glass slide.
- Used in the observation of stained Chromosome with Giemsa stain.
- Used for observation of microbial cells.

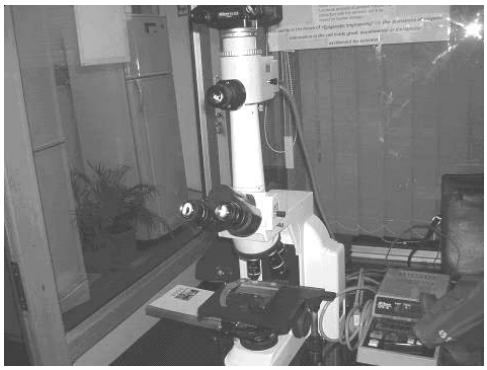

FIGURE 1.6

Centrifuge

REMI, INDIA:

R8C Remi® Laboratory Centrifuge:

- Used for centrifugation of Microbial/Plant/Animal tissue and cell for the formation of pellet during harvesting and reseeding procedure.
- Cells pellets generally formed at 1000 rpm for 10 min. in this centrifuge.
- Refrigerated centrifuge was basically used in the RNA isolation purpose and Separation of temperature sensitive cell organelles/Bio molecules etc. with the help of centrifugal force.

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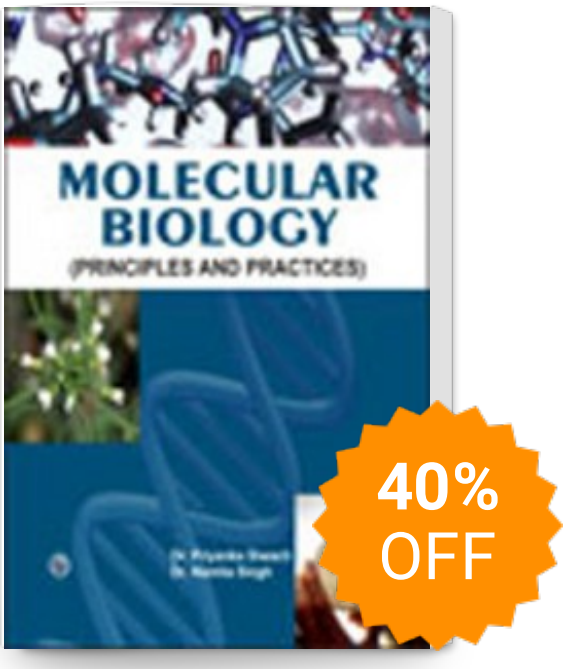

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