Faculty of Science

Laboratory Manual

Developmental Biology

Bachelor of Biotechnology (Hons.)

Edited By: Dr. Tapash Rudra

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LINCOLN UNIVERSITY COLLEGE
Wisma Lincoln, No. 12,14,16 & 18,
Jalan SS 6/12, Off Jalan Perbandaran
47301 Petaling, Jaya,
Selangor Darul Ehsan, Malaysia
Tel.: +603-7806 3478
Fax: +603-7806 3479
Toll Free: 1-300-880-111
E-mail: lucp@lincoln.edu.my
info@lincoln.edu.my
Web: www.lucp.net
www.lincoln.edu.my

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Developmental Biology

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LINCOLN UNIVERSITY COLLEGE
FACULTY OF SCIENCE (DEPARTMENT OF BIOTECHNOLOGY)
LABORATORY SAFETY RULES

The following rules must be obeyed by all students in the science laboratory of the faculty. Wilful or repeated in advertent non-compliance may result in dismissal or suspension from the laboratories

- No entry without permission:
  - Outsiders are not allowed to enter the laboratory without permission.
  - No student is allowed to enter the laboratory unless permission has been given by a laboratory assistant or a lecturer.

- At work in the laboratory:
  - No experiment may be attempted without the knowledge and permission of a lecturer.
  - Students must wear shoes in the laboratory. Students wearing slippers or sandals are not allowed to work in the laboratory.
  - Lab coat must be worn at all times during practical work in the laboratory.
  - Do not mouth pipette chemicals.
  - Do not eat or smoke in the laboratory.
  - Do not taste any chemicals, including dilute solutions. If any acid or alkali accidentally enters your eyes or mouth, wash immediately with plenty of tap water. Inform your lecturer, and seek medical attention if necessary.
  - Paper should be used to light up the Bunsen burners.
  - Used match sticks, filter papers, and other solid waste must never be thrown into the sinks. They must be thrown into the dustbins provided. Lighted match sticks and smoldering materials must be extinguished with tap water before thrown in to the dustbins.
  - Any equipment broken or damaged must be reported to the laboratory assistant.

- Before leaving the laboratory:
  - All the equipment and benches must be cleaned at the end of each practical session.
  - Wash hands and arms with soap and water before leaving the laboratory.
  - No student is allowed to take away any chemicals, equipment or other property of the laboratory.

INTRODUCTION

1. The Scientific Method

- Making observations
- Generating hypotheses
- Making predictions
- Designing and carrying out experiments
- Constructing scientific models

2. Practical Exercises

To get the most out of the practical exercises, you need to follow carefully the instructions given. These instructions have been designed to provide you with the experience in the following skills:

- Following instructors
- Handling apparatus
- Having due regard for safely
- Making accurate observations
- Recording results in an appropriate form
- Presenting quantitative results
- Drawing conclusions

3. Following Instructions

Instructions are provided in the order in which you need to carry them out. We would advise that before carrying out the instructions, you read through the entire exercise. This will help you to remember what you have learned.

Each practical exercise in the book begins with a few lines describing its purpose in most cases the following headings are also used:

- Procedure-numbered steps that need to be carried out.
- For consideration -some questions to help you think carefully about the results you have obtained.
- Materials-a list of the apparatus, chemicals and biological materials you need.

4. Handling apparatus

Biologists need to able to use many different types of apparatus, for example, photometers (to measure water uptake by plants), respirometers (to measure oxygen uptake or carbon dioxide production), Petri dishes (for plating out bacteria and other microorganisms) and the light microscope (to magnify specimens). Many of the practical exercises are designed to help you derive the maximum benefit from a piece of apparatus.

5. Having Due Regard for Safety

Surveys have been shown that science laboratories are among the safest places to be. Nevertheless, this is no cause for complacency.

- Always move slowly and carefully in a laboratory.
- Never put your fingers in your mouth or eyes after using chemicals or touching biological specimens until you have washed your hands thoroughly with soap and warm water, and dried them.
- Make sure glass objects (e.g, thermometers, beakers) cannot roll off tables or be knocked onto the floor.
- Wear safely goggles whenever there is a risk of damage to the eyes.

<u>Situations of risk include:</u>

- Heating anything with a Bunsen burner (even heating water has its dangers')
- Handling many liquids, particularly those identified as corrosive, irritant, toxic or harmful

- Handling corrosive or irritant solids
- Some dissection work
- Allow Bunsen burners, tripods, gauzez and beakers to cool down before handling them.
- Never allow your own body fluids (especially blood and saliva) to come into contact with someone else, or theirs into contact with you.
- Keep long hair tied back and do not wear dangly earrings.
- Do not allow electrical equipment to come into contact with water.
- If you are unsure how to carry out a scientific procedure, ask.
- Make sure you understand why you are going to do something before you do it.
- Wear a lab coat when using chemicals or handling any biological specimens.
- Follow exactly agreed procedures with regard to cuts, burns, electric shocks and other accidents (e.g. with chemicals).
- Follow exactly all specific safely instructions given in this book or provided by your teacher for particular practical exercises (e.g. use of gloves, disinfection)

With practice, these procedures should become second nature to you. They will enable you to carry out practical work in safety.

6. Making Accurate Observations

In most cases the practical exercise will make it clear what you need to observe, e.g. the time taken for a certain volume of gas to be evolved or the width of a sample cells. Ensure that you know how to use any necessary equipment before starting practical. Think carefully about the precision with which you will make your observations.

7. Recording Results in an Appropriate Form

Results can be recorded in various ways. Often it is helpful to record raw data in a table. Most data will be in the form of numbers, i.e. they will be quantitative data (also known as numerical data). However, some data, e.g. flower colour, will be qualitative data.

One form in which some biological findings can be recorded is a drawing. You don't need to be professional artist to make worthwhile biological drawings. If you follow the following guidelines, a drawing can be of considerable biological value:

- Ensure that your completed drawing will cover at least a third of A4 page.
- Plan your drawing so that the various parts are is proportion and will not be drawn too small. Small marks to indicate the length and breadth of the drawing are a great help in planning and a faint outline can be rapidly drawn to show the relative positions of the parts.
- The final drawing should be made with clean, firm lines using a sharp HB pencil and, if needed, a good quality eraser (not a fluid).If important details are too small to be shown in proportion, they can be put in an enlarged drawing at the side of the main drawing.
- Avoid shading and the use of colour unless you are an excellent artist and they really help, for example when drawing soil profiles.
- When drawing structures seen with the naked eye or hand lens, use two lines to delineate such things as blood vessels and petioles. This will help you to indicate the relative widths of such structures.
- When drawing low power plan drawings from the light microscope, do not attempt to draw individual cells-just different tissues.
- When drawing plant cells at high power under the light microscope, use two lines to indicate the width of cell walls, but a single line to indicate a membrane.
- Always put a scale on each drawing.

8. Presenting Quantitative Results

Presentation of data is all about using graphs or other visual means to make it easier to see what your results tell you. The following four ways of presenting data are the most frequently used in biology: line graphs, bar charts, histograms and scatter graphs (Figure 1).

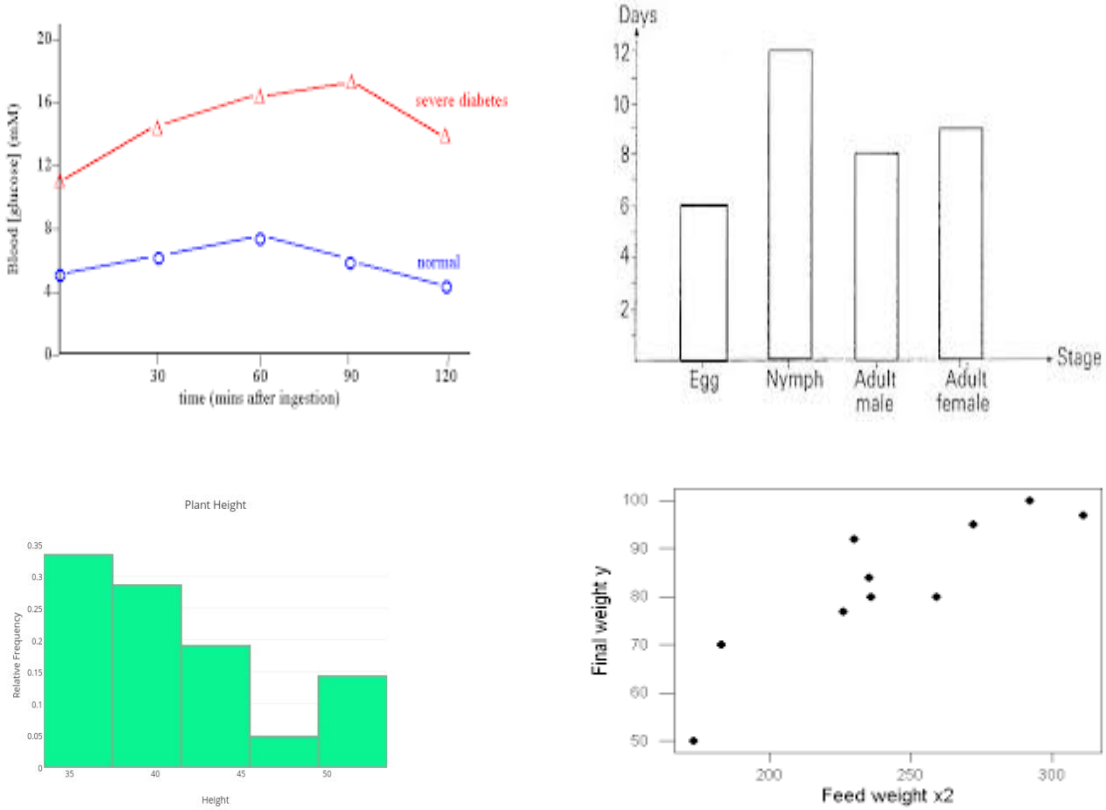


Figure 1: Line graphs, bar charts, histograms and scatter graphs

9. Drawing Conclusions

Finally, you will need to draw conclusions. If your practical exercise has involved the testing of a hypothesis, for example that the enzyme pepsin works better at low pH than in neutral or alkaline conditions, your conclusion should indicate whether the hypothesis has been refuted (i.e. shown not to be the case) or supported. Of course, even if your hypothesis has been supported, it doesn't mean that it has been confirmed with 100% certainty- in other words it isn't proved. Science proceeds more by showing that certain ideas are wrong than by showing that others are right (think about that!). Your conclusion might therefore include further ways of testing the original hypothesis, or might raise new possibilities to be investigated.

Often you will only be able to arrive at your conclusions after statistically analysing your data.

10. Writing a Scientific Lab Report

Title

- Communicate the subject investigated in the paper.

Introduction

- State the hypothesis.
- Give well-defined reasons for making the hypothesis.
- Explain the biological basis of the experiment.
- Cite sources to substantiate background information.

- Explain how the method used will produce information relevant to your hypothesis.
- State a prediction based on your hypothesis. (If the hypotheis is supported, then the results will be.)

Materials and Methods

- Use the appropriate style.
- Give enough detail so the reader could duplicate your experiment
- State the control treatment, replication and standardized variables that were used.

Results

- Summarize the data (do not include raw data).
- Present the data in an appropriate format (table or graph).
- Present tables and figures neatly so they are easily read.
- Label the axes of each graph completely.
- Give units of measurement where appropriate.
- Write a descriptive caption for each table and figure.
- Include a short paragraph pointing out important results but do not interpret the data.

Discussion

- State whether the hypothesis was supported or proven false by the results, or else state that the results were inconclusive.
- Cite specific results that support your conclusions.
- Give the reasoning for your conclusions.
- Demonstrate that you understand the biological meaning of your results.
- Compare the results, with your predictions and explain any unexpected results.
- Compare the results to other research or information available to you.
- Discuss any weaknesses in your experimental design or problems with the execution of the experiment.
- Discuss how you might extend or improve your experiment.

Conclusion

- Restate your conclusion.
- Restate important results.

Literature Cited

- Use the proper citation form in the text.
- Use proper citation form in the Literature Cited section.
- Refer in the text to any source listed in this section.

Acknowledgement

- State any appropriate acknowledgement that you think is necessary.

Practical 1

Title: Determination of morphogenesis in vertebrate embryo

Objective:

After completing the practical, you will be able:

1. To assess how morphogenesis happens in vertebrates

Introduction:

The chicken embryo is a staple educational tool in developmental biology. The availability and similarity with mammalian embryo, help to shape our present understanding of embryology. The development of the chick begins in the single cell formed by the union of two parental cells, egg and sperm, in the process known as fertilization. In birds, fertilization occurs about 24 hours before the egg is laid. The newly formed single cell begins to divide into 2, then 4, 8, 16, 32 and so on. At the time of laying, hundreds of cells are grouped in a small, whitish spot (the blastoderm or germinal disc) that is easily seen on the surface of the yolk. When the egg is laid and cools, division of the cells ceases. After the egg is laid, cooling the egg after the egg is laid does not result in the death of the embryo. It may resume its development after several days of rest if it is again heated by the hen or in an incubator.

Materials:

1. 70% alcohol
2. Carborundum disc
3. Ringer's solution
4. Forceps
5. Glass needle
6. Paraffin film
7. Micro tacks
8. Colored soft glass
9. Nichrome wire

Procedure:

1. A three-day embryo is located by candling and its position is marked on the shell.
2. The shell that was marked was wiped with a 70% alcohol by using cotton.
3. A hole is drilled in the small end of the egg to allow removal of the albumen.
4. 2 ml of albumen are removed to lower the embryo so that it's extra–embryonic membranes will not stick to the shell.
5. A window is cut in the shell with a rotating carborundum disc.
6. Ringer's solution is used to wash away the shell dust and to moisten the shell membrane.
7. The shell portion that has been cut was taken out carefully by using forceps.
8. The limb bud is exposed for operation by teasing away the overlaying chorion and amnion.
9. A wing tip is severed from a 3 – day donor embryo by means of glass needle.
10. The wing bud was then placed into the Ringer's solution.
11. After an operation, the window is closed. A warm iron is touched to paraffin film to seal it to the shell. The embryo is the returned to the incubator to continue development.
12. Glass Operating Needles Protocol
13. Glass operating needles and micro tacks are needed for microsurgery and grafting experiments.
14. The glass operating needle consists of a fine fiber drawn from deeply colored soft glass.

15.The glass fiber is fused to a glass handle.
16.The point of the operating needle is melted in an electrically heated nichrome wire, then drawn out and given an L – shaped tip.
17.Micro tacks are made by the same method from the fine fibers of colored glass.

Observation/Results:

Questions:

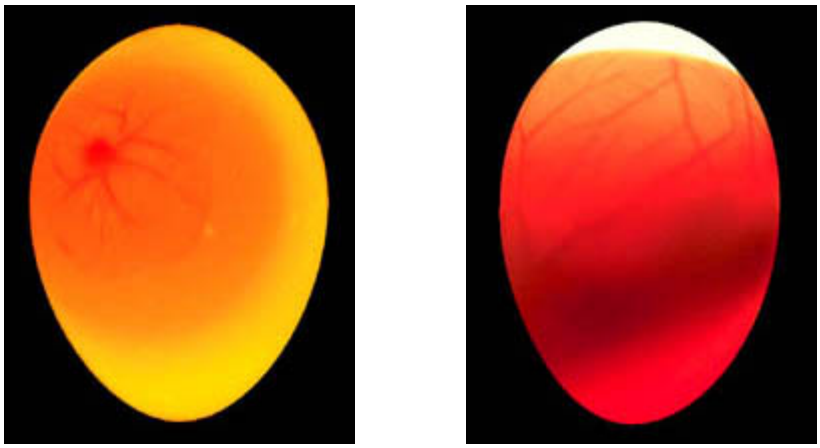
1. Explain the process of morphogenesis of vertebrate embryo.
2. Explain the development of embryonic layers during morphogenesis.

Practical 2
Title: Isolation of shell-less chick embryo

Objective:
After completing the practical, you will be able:
1. To assess how morphogenesis happens in vertebrates

Introduction:

Special temporary organs or embryonic membranes formed within the egg, both to protect the embryo and to provide for its nutrition, respiration, and excretion. These organs include the yolk sac, amnion, and allantois. The yolk sac supplies food material to the embryo. The amnion, by enclosing the embryo, provides protection. The allantois serves as a respiratory organ, gets minerals from the shell, and handles waste. These temporary organs function within the egg until the time of hatching. Figure 1 shows embryo at 3 and 9.



Embryo: day 3 Embryo: day 9

Figure 1: Embryo at day 3 and 9

Materials:
- 70% ethanol
- Sterile petri dish

Procedure:

1. Squirt a particular section of the shell and treated with 70% ethanol. It is then allowed to dry in an incubator.
2. A cradle is being made at the middle of the shell by using a plastic wrap. Cover it with a lid or a sterile petri dish.
3. Wipe the 3-day egg with 70% ethanol. Allow to be placed in incubator for 5-10 minutes to allow embryo to rotate to the top side.
4. Remove from incubator and uncover the lid. Crack the lower side of the shell using the edge of the finger bowl and cover it again with a lid or sterile petri dish and return back into the incubator at 37 °C.

Note: Obviously the embryo will develop best if the yolk is not broken. Warm, incubated eggs break more easily than refrigerated ones.

5. Observe periodically to watch organ development (organogenesis) and extra embryonic membrane.

Observations/ Results:

Questions:

1. Explain the process of organogenesis in vertebrate embryo.
2. Explain the development of embryonic layers during organogenesis.

Practical 3
Title: Assessment of early vertebrate embryo (Chick)

Objective:
After completing the practical, you will be able:
1. To observe the *in vitro* development of early chick embryo

Introduction:

The development of a complex organism from a single cell, the fertilised egg, has fascinated people for centuries. Embryo development is highly reproducible and exquisitely regulated. How is it that all tissues and organs form in the right places and at the right time? How is the development of different organ systems coordinated, so that they all fit together correctly at the end? It is challenging to study development, because many embryos are small or inaccessible. The chick embryo is a popular model system with many experimental advantages, which include classic 'cut and paste' experiments and mechanistic gene function analyses. The combination of micromanipulations with gain- or loss-of-function is particularly powerful. The recent development of transgenic lines and advanced imaging techniques ensure that the chicken remains an attractive model system, which will continue to make major contributions to our understanding of molecular and cellular mechanisms controlling developmental processes.

Key Concepts
- The chick embryo is easy to access and observe and is therefore an attractive model to study developmental processes.
- In the early stages, chick embryo morphology is very similar to human, both are amniotes and their development is very similar.
- The chicken and human genomes share considerable homology.
- Classic embryological manipulations, such as tissue ablations and tissue grafts, provide information about the contribution of particular cell populations to various organs. These experiments have also told us how cells and tissues influence each other in their developmental decisions.
- The molecular and cellular basis for many developmental processes and phenomena were first described in the chick, including limb patterning, neural crest migration, dorso-ventral neural tube patterning, blood vessel formation, somite segmentation and left–right asymmetry.
- Experiments in chicken, which examine the function of genes, have helped elucidate the underlying mechanisms of human genetic diseases and provide a basis for testing novel therapies.
- Time-lapse video microscopy can be used to image live chick embryos, either in ovo or ex ovo using embryo culture.
- We can use chick embryos to visualise complex processes, including cell migration, cell–cell communication, cell differentiation and tissue morphogenesis, in an amniote system.

Materials:

1. Sterile petri dish
2. Ringer's solution
3. Pipette

Procedure:

1. Use sterile technique. Ringer's solution in sterile petri dish and place in incubator for 37°C.
2. Crack a 4-day old egg into a sterile petri dish. Alternatively, open blunt end of egg and remove the shell membrane.
3. Use a disposable pipette to remove some portion of the albumin so that the top half of the yolk is uncovered.
4. Place filter paper around the blastodisc and cut around it.
5. Transfer into Ringer's solution.
6. Stage embryo and gently wash away adherent yolk.
7. Transfer to albumin dish filter paper upside down.
8. Incubate at 37°C.

Observations/ Result:

Practical 4
Title: Isolation of Zebra fish embryo

Objective:

After completing the practical, you will be able:

1. To learn and acquire skills to deal with the separation of vertebrate embryos

Introduction:

Zebrafish embryos are a tremendously under-utilized source of stem, progenitor, and differentiating cells for exploring questions of single cell heterogeneity and molecular regulation of cellular identities during development. Their highly stereotyped, *ex vivo* development and ease of genetic manipulation make them an excellent model system for this approach. Specifically, a major limitation to interpretation of single cell gene expression data is that reliable identification of novel intermediate cell states during development requires very careful timing of tissue collection. This is necessary to ensure that heterogeneity between captured cells represents heterogeneity within a tissue at a single time point rather than heterogeneity in gene expression presented by age-dependent cell differentiation. Compared to mice, zebrafish embryo development may be precisely synchronized across a large number of embryos. Additionally, with large clutch sizes, zebrafish embryos can be used as an abundant source of stem and progenitor cells.

Materials

- Fish tank
- Male/ Female zebra fish
- Pipette
- Aquarium pump
- Air stone
- Siphon
- Filter mesh
- Microscope

Procedure:

1. Zebra fish will mate and deposit fertilized eggs on the bottom of the tank at 'dawn'. They can be accustomed to lay at any convenient time by keeping the room dark and lighting the tank with a timer.
2. The maximum embryo production is obtained with a ratio of 1 male for every 2 females, with a group of 20-25 fishes.
3. The night before embryo collection, vacuum the tank well with a siphon to remove debris several hours after the last feeding.
4. Deposit a layer of washed marbles to cover the entire bottom surface of the tank. Do not disturb for the first 30 minutes after 'dawn' to allow fish to mate.
5. Embryos can be collected once a week, with best results from tanks kept on a regular schedule. Collect embryos and fertilized eggs from between the marbles with a siphon and collect with a fine fry net or mesh filter.
6. Transfer the net to a glass dish full of tank water and examine the embryos with a dissecting microscope at low magnification.
7. Transfer healthy embryos to a clean glass petri dish containing with large-bore glass Pasteur pipets.
8. Discard any that are cloudy or ruptured. Keep embryos at 28°C.

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15. Transfer healthy embryos to a clean glass petri dish containing with large-bore glass Pasteur pipets.
16. Discard any that are cloudy or ruptured. Keep embryos at 28°C.

Observations/Results:

Notes

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