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Welcome to BABS1201 Molecules, Cells and Genes

This course aims to introduce you to the basic concepts of modern biology, and to develop your skills in scientific analysis and critical thinking – skills that will be useful in science and other careers.

The course consists of two interconnected learning components: a lecture series and a laboratory program. These classes sequentially address cell structure, cell function and genes. The laboratory program comprises both practical and theoretical components. You will also be directed to electronic resources (Mastering biology) that revise and reinforce the concepts covered in these components.

To be ready for your first classes, ensure that you have purchased or printed a copy of the Course Manual (available from the UNSW Bookshop or the course Moodle site). In preparation for your lectures, you can download the notes from Moodle and can read relevant material from the course textbook (available from the UNSW Bookshop, as an ebook or from the library).

Prior to your laboratory classes, you need to purchase a laboratory coat (available at various stores on campus) and, before Practical 1, complete an online Health and Safety quiz. You must come to your timetabled lab class wearing closed shoes and bring a copy of the Course Manual and appropriate stationery. As electronic resources will be used in class, you are encouraged to bring a laptop, tablet or similar device. If you do not have access to one, you may borrow a tablet from the BSB Office.

To ensure you are organised for the year, look ahead in this outline to the due dates of your assignments and enter them into your diaries. Details of the assignments are included in the Course Manual and further information will be posted on Moodle as the due dates approach.

If you have a question that has not been addressed in the resources provided, please post on the course Moodle forum. If your question is sensitive or of a personal nature, email BABS1201@unsw.edu.au, with is directed to both of us and the course administrator. Our laboratory classes are run relatively informally, so you are welcome to ask questions and discuss the material with your demonstrators in class as needed.

We hope your study of biology this session will be interesting, enjoyable and rewarding.

We look forward to teaching you.

Rebecca and John

Course Convenors
GENERAL

• I have a question about the course. Where do I find the answer?

1. Look in this manual. Use the table of contents on page 1 to help you.

2. Check the BABS1201 Moodle site: https://moodle.telt.unsw.edu.au/login/index.php

3. Post your question to the 'Discussion Forum' in the BABS1201 Moodle site.

4. Email your question to BABS1201@unsw.edu.au (this option is especially useful when your question is of a personal or sensitive nature). ALWAYS include your full name and student number in ALL email correspondence, and send from your UNSW email account

5. Take the time to clearly word your query.

• How do I find answers to questions about specific lecture material?

1. Read through the corresponding lecture notes whilst listening to the lecture audio recording (lecture notes and Echo360 lecture recordings can be accessed through the BABS1201 Moodle site). The lecturer may have answered your question during the lecture.

2. Refer to the corresponding reference(s) provided by the lecturer. If references are not provided or are not related to your question, use the index of a biology text book (even if it is not the recommended text for the course) to search for information on the topic of interest. There are also copies of the recommended text in the library.

3. Search the internet using appropriate key terms.

4. Post your question to the 'Discussion Forum' in the BABS1201 Moodle site.

5. Email your question to BABS1201@unsw.edu.au, including your full name and student number, as well as the full details of the exact lecture to which your question refers.

• How do I contact my demonstrator? Can I email them?

No, you cannot directly contact your demonstrator. You can instead post your question to the Moodle Discussion Forum as some demonstrators help to answer questions in the forum. Also, in this way, all students will have a chance to see the answer. If the matter is not on the content of the course eg. it relates to your attendance, you can email your question (clearly indicating your laboratory class and demonstrator name) to BABS1201@unsw.edu.au, and, if necessary, it will be forwarded to your demonstrator. Otherwise, your question can be addressed directly by your demonstrator during the next laboratory class.

• Will I have access to past BABS1201 exam papers?

No, past BABS1201 exams are not available to students. Sample questions are instead provided and the Mastering Biology activities are particularly useful for exam preparation.

ABSENCES AND ASSESSMENTS

• I missed a lab class. What should I do?

Attendance at all lab classes is compulsory. If you miss a lab class due to illness or some other unavoidable circumstance that can be explained using professional documentation (e.g. a medical certificate), you should provide your demonstrator with this documentation the following week.
• I missed the mid-session exam. What should I do?
If you miss the mid-session exam due to illness or some other unavoidable circumstance that can be explained using professional documentation (e.g. a medical certificate), you should apply for Special Consideration online (see page 19 for instructions). You will be notified of the outcome of your application before the end of session through myUNSW and/or your UNSW email account.

• I could not submit my assessment on time. What should I do?
If you cannot submit your essay or practical report by the due date due to illness or an unavoidable circumstance that can be explained using professional documentation (e.g. a medical certificate), you should apply for Special Consideration online (see page 19 for instructions) and submit the assessment item as soon as possible. You will be notified of the outcome of your application before the end of session through myUNSW and/or your UNSW email account. If you do not submit an assessment item by the submission deadline and you do not have a valid excuse, the appropriate late penalties will be applied to your final mark for that assessment item.

• I missed (or was sick during) the final exam. What should I do?
If you miss the final exam due to illness or some other unavoidable circumstance that can be explained using professional documentation (e.g. a medical certificate), you should apply for Special Consideration online (see page 19 for instructions). If you were sick DURING the exam, you should obtain a medical certificate on the day of the exam and apply for Special Consideration online (see page 19 for instructions). You will be notified of the outcome of your application and details of the supplementary examination (if applicable) through your UNSW e-mail account. See page 19 for details of the supplementary exam.

Please note that if you are offered a supplementary exam, you will only be given one opportunity to attend this, unless there are exceptional circumstances.
<table>
<thead>
<tr>
<th>Week No.</th>
<th>Week Commencing</th>
<th>LECTURE A</th>
<th>LECTURE B</th>
<th>Laboratory</th>
<th>Assessments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27-Feb</td>
<td>1. What is BABS1201? – RLB/JW</td>
<td>2. Life – RLB</td>
<td>Introduction and Safety</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>20-Mar</td>
<td>7. Cellular Transport - VS</td>
<td>SESSION TEST</td>
<td>Prac 3. Cell Structure III Differences in cell types</td>
<td>Mastering biology Quiz 1 (1%)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Mid-Session Test 1 (15%)</td>
</tr>
<tr>
<td>6</td>
<td>3-Apr</td>
<td>11. Metabolism II - JW</td>
<td>PUBLIC HOLIDAY</td>
<td>No Lab</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>17-Apr</td>
<td></td>
<td></td>
<td>MID-SESSION BREAK</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>24-Apr</td>
<td>PUBLIC HOLIDAY</td>
<td>14. Cell Division and Reproduction - LLM</td>
<td>No lab</td>
<td>Mastering biology Quiz 2 (1%)</td>
</tr>
<tr>
<td>10</td>
<td>8-May</td>
<td>17. The Polymerase Chain Reaction - LLM</td>
<td>18. Mutation - LLM</td>
<td>Prac 7. Genes I Mitosis and cell division</td>
<td>Enzymes Report Due (15%)</td>
</tr>
<tr>
<td>11</td>
<td>15-May</td>
<td>SESSION TEST 2</td>
<td>19. Mendel's Laws of Heredity - PW</td>
<td>Prac 8. Genes II Genetics inheritance</td>
<td>Mastering biology Quiz 4 (1%) Mid-Session Test 2 (15%)</td>
</tr>
<tr>
<td>13</td>
<td>29-May</td>
<td>22. Exam information and tips - RLB/JW</td>
<td></td>
<td>No lecture</td>
<td>Biological modelling (10%)</td>
</tr>
</tbody>
</table>

JW: John Wilson, RLB: Rebecca LeBard, LLM: Louise Lutze-Mann, VS: Vladimir Sytnyk, PW: Paul Waters
<table>
<thead>
<tr>
<th>Course Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Course Code</strong></td>
</tr>
<tr>
<td><strong>Course Name</strong></td>
</tr>
<tr>
<td><strong>Academic Unit</strong></td>
</tr>
<tr>
<td><strong>Level of Course</strong></td>
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<tr>
<td><strong>Units of Credit</strong></td>
</tr>
<tr>
<td><strong>Assumed Knowledge, Prerequisites or Co-requisites</strong></td>
</tr>
<tr>
<td><strong>Hours per Week</strong></td>
</tr>
<tr>
<td><strong>Number of Weeks</strong></td>
</tr>
</tbody>
</table>

| Course Convenors | Dr Rebecca LeBard  
Room 103, Level 1, Biological Sciences Building (eastern end)  
тел. (02) 9385 2026; Meetings by appointment only.  
Email: r.lebard@unsw.edu.au |
|------------------|-----------------------------------------------------------------|
|                  | John Wilson  
Room G27 (inside the BSB Office), Biological Sciences Building  
тел. (02) 9385 8156; Meetings by appointment only.  
Email: j.e.wilson@unsw.edu.au |

| Administrative Support (enrolment queries, academic advise etc) | Dean Lovett  
BABS1201 Course Administrator  
E-mail: BABS1201@unsw.edu.au  
BABS/SOMS/BEES (BSB) Student office  
Room G27, Ground floor, Biological Sciences Building  
tел. (02) 9385 8047; Opening Hours: 9:00am – 4:30pm, Mon-Fri.  
Email: BABStudent@unsw.edu.au |
### Course Resources

<table>
<thead>
<tr>
<th>Recommended Textbook</th>
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</table>

<table>
<thead>
<tr>
<th>Course Web Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>BABS1201 uses Moodle as an on-line course management software. This website contains background information, links to resources, lecture notes, and discussion forums. Once you are enrolled in BABS1201, you can access the Moodle site at: <a href="https://moodle.telt.unsw.edu.au/login/index.php">https://moodle.telt.unsw.edu.au/login/index.php</a> Your username is your student number preceded by a lower-case z e.g. z1234567. Your password is your zpass.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Course Manual</th>
</tr>
</thead>
<tbody>
<tr>
<td>A course manual is required and may be purchased from the UNSW Bookshop or downloaded from the BABS1201 Moodle site. Please Note: If you choose to print your own copy, make sure that you bind it or file it in a folder and bring the COMPLETE manual to all lab classes.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Practical Class Requirements</th>
</tr>
</thead>
</table>
| For all practical classes students are required to bring:  
  • The complete BABS1201 Molecules, Cells & Genes course manual. Read the instructions in advance for each practical.  
  • A laboratory coat and closed shoes. This is required by Health and Safety (HS) regulations, and you will not be permitted to participate in practicals if you are inappropriately clothed. If you have long hair, you must also wear it tied back during practical classes. Laboratory coats can be purchased from various stores on campus.  
  • Material for recording your observations and findings as appropriate for each class. These items include: a pen, an HB pencil, eraser, ruler and lined paper for written observations and plain paper for drawings. |
## Course Assessment Schedule & Summary

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Brief Description</th>
<th>Due Date</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Online Laboratory Safety Quiz</strong></td>
<td>This is a COMPULSORY online laboratory safety quiz that is accessed through the BABS1201 Moodle site. You must complete this quiz with a mark of 100% BEFORE your Week 2 practical class.</td>
<td>Practical 1 Week 2</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>B. Session Test 1</strong></td>
<td>Duration: 40 minutes. <strong>Format</strong>: multiple choice questions. <strong>Content</strong>: all theory and practical material from Weeks 1-3 (inclusive). <strong>Venue</strong>: your enrolled lecture venue. <strong>Time</strong>: your enrolled lecture time.</td>
<td>Wed or Fri Week 4</td>
<td>15%</td>
</tr>
<tr>
<td><strong>C. Scientific Literature Essay</strong></td>
<td><strong>ESSAY</strong>: You must prepare and submit your essay in class (hard copy) and online via the Turnitin plagiarism checking software in Moodle.</td>
<td>Practical 3 Week 5</td>
<td>10%</td>
</tr>
<tr>
<td><strong>D. Team Enzyme Project</strong></td>
<td>1. <strong>EXPERIMENTAL PROTOCOL</strong>: In teams of two students, you are firstly required to design an experimental protocol that investigates enzyme structure and/or function. Your team’s final protocol will be submitted to your demonstrator in class.</td>
<td>Practical 4 Week 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2. <strong>EXPERIMENTAL METHOD AND NOTES</strong>: As a team, you will carry out the procedures outlined in your final experimental protocol during Practical 5. You must record all data obtained and any observations made during this class in your manual. At the end of the class, your demonstrator will assess your written experimental notes.</td>
<td>Practical 6 Week 9</td>
<td>15%</td>
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<tr>
<td></td>
<td>3. <strong>FINAL REPORT</strong>: Each team member is required to write their own scientific report on the experimental procedures and outcomes of their project according to the guidelines provided. A hard copy of your report is to be submitted to your demonstrator at the commencement of your revision lab class in Week 10.</td>
<td>Practical 7 Week 10</td>
<td></td>
</tr>
<tr>
<td><strong>E. Session Test 2</strong></td>
<td><strong>Duration</strong>: 40 minutes. <strong>Format</strong>: multiple choice questions. <strong>Content</strong>: all theory and practical material from Weeks 4-10 (inclusive). <strong>Venue</strong>: your enrolled lecture venue. <strong>Time</strong>: your enrolled lecture time</td>
<td>Mon or Tue Week 11</td>
<td>15%</td>
</tr>
<tr>
<td><strong>F. Mastering biology</strong></td>
<td>Online Mastering biology tasks designed to provide you with formative feedback on how you are progressing in the course. Each task is a short quiz (15-30 minutes) worth 1% of your final assessment for the course. Preparation time is allocated during some practical classes, and Mastering biology is also accessible via Moodle. You are able to discuss the questions with your peers in the class, the aim is to assist you in learning. You have until the end of the week following your lab to complete the quiz.</td>
<td>11:59pm Friday Weeks 4, 8, 9, 11 and 12</td>
<td>5%</td>
</tr>
<tr>
<td><strong>G. Biological modelling</strong></td>
<td>In teams of four you will create a model explaining a biological system and present it in class. As part of this assessment you will need to document group meetings and progress on a template downloaded from Moodle. Further details will be posted on Moodle after the groups are formed. <strong>Venue and time</strong>: your enrolled laboratory classroom and time.</td>
<td>Practical 10 Week 13</td>
<td>10%</td>
</tr>
<tr>
<td><strong>H. Final Theory Exam</strong></td>
<td><strong>Duration</strong>: 2 hours. <strong>Format</strong>: the front page of the exam paper, detailing the format (including the number and type of questions) will be available uploaded to Moodle after submission to the exams branch. <strong>Content</strong>: all theory and practical material from Weeks 1-13 of session. <strong>NOTE</strong>: The final exam is a COMPULSORY assessment and must be attempted in order to satisfy the requirements for passing the course.</td>
<td>June (Date to be advised by exams branch)</td>
<td>30%</td>
</tr>
</tbody>
</table>
# Mastering Biology Assessment Schedule and Summary

<table>
<thead>
<tr>
<th>Quiz</th>
<th>Topic</th>
<th>Median Time</th>
<th>Date Available Online</th>
<th>Date Due</th>
</tr>
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<tbody>
<tr>
<td>Quiz 1: Prokaryotic Versus Eukaryotic Cell Structure</td>
<td>5 min</td>
<td>13 March</td>
<td>24 March</td>
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<tr>
<td>Activity: Prokaryotic Cell Structure and Function</td>
<td>5 min</td>
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<tr>
<td>Activity: Review: Animal Cell Structure and Function</td>
<td>9 min</td>
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<tr>
<td>Total Time</td>
<td>14 min</td>
<td></td>
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<tr>
<td>Quiz 2: Osmosis and Diffusion</td>
<td>2 min</td>
<td>10 April</td>
<td>21 April</td>
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<tr>
<td>Activity: Selective Permeability of Membranes</td>
<td>2 min</td>
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<tr>
<td>Activity: Diffusion</td>
<td>1 min</td>
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<tr>
<td>Activity: Diffusion and Osmosis</td>
<td>6 min</td>
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<tr>
<td>Activity: Facilitated Diffusion</td>
<td>1 min</td>
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<tr>
<td>Activity: Osmosis and Water Balance in Cells</td>
<td>4 min</td>
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<tr>
<td>Activity: Active Transport</td>
<td>2 min</td>
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<tr>
<td>Total Time</td>
<td>15 min</td>
<td></td>
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<tr>
<td>Quiz 3: Photosynthesis</td>
<td>3 min</td>
<td>17 April</td>
<td>28 April</td>
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<tr>
<td>Activity: Overview of Photosynthesis</td>
<td>3 min</td>
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<tr>
<td>Activity: The Sites of Photosynthesis</td>
<td>1 min</td>
<td></td>
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<tr>
<td>Tutorial: Energy Flow in Plants - Concept Map</td>
<td>3 min</td>
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<tr>
<td>Activity: The Light Reactions</td>
<td>4 min</td>
<td></td>
<td></td>
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<tr>
<td>Activity: Photosynthesis</td>
<td>5 min</td>
<td></td>
<td></td>
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<tr>
<td>Activity: The Calvin Cycle</td>
<td>3 min</td>
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<tr>
<td>Total Time</td>
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<tr>
<td>Quiz 4: Mitosis and Meiosis</td>
<td>8 min</td>
<td>8 May</td>
<td>19 May</td>
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<tr>
<td>Activity: Mitosis and Cytokinesis Animation</td>
<td>8 min</td>
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<td></td>
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<tr>
<td>Activity: The Cell Cycle</td>
<td>3 min</td>
<td></td>
<td></td>
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<tr>
<td>Activity: Meiosis Animation</td>
<td>10 min</td>
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<td>Total Time</td>
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<tr>
<td>Quiz 5: DNA Structure and Replication</td>
<td>8 min</td>
<td>15 May</td>
<td>26 May</td>
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<tr>
<td>Activity: DNA and RNA Structure</td>
<td>8 min</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Activity: DNA Replication: A Closer Look</td>
<td>4 min</td>
<td></td>
<td></td>
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<tr>
<td>Tutorial: DNA Replication</td>
<td>2 min</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Activity: DNA Replication: A Review</td>
<td>4 min</td>
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<td></td>
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<tr>
<td>Activity: DNA Replication: An Overview</td>
<td>3 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity: DNA Synthesis</td>
<td>4 min</td>
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<tr>
<td>Total Time</td>
<td>25 min</td>
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</table>

**Each Quiz is Worth 1% of Your Final Assessment (5% Total).**

Quizzes are not timed, but each must be completed by the due date.
## Course Outline

The course, Molecules, Cells and Genes encompasses four major themes. These themes are not presented in turn, but rather will be presented in an integrated fashion.

| Theme 1: Thinking like a scientist | This theme introduces the skills of scientific thinking, including how to decide what is true or plausible, and how scientists communicate. It also exposes you to cutting edge research being conducted at UNSW. Lectures and practical classes on this theme are interspersed through the session, enabling you:  
- To comprehend that science is a never-ending exploration, and that knowledge is provisional.  
- To identify the principal characteristics of scientific evidence.  
- To understand how scientists approach the investigation of a topic.  
- To communicate the principles of scientific findings to other scientists. |
|---------------------------------|---------------------------------------------------------------------------------------------------|
| Theme 2: Cell biology and cell architecture | This theme describes the principal types of living cells, the key components of cell structure, their functions, and how they relate to each other. Lectures and practical classes on this theme should enable you:  
- To understand the evolutionary origins of life, and of the diversity of life.  
- To identify the different types of living cells, and the main similarities and differences between them.  
- To explain how different cell types are identified.  
- To describe important cell structures and relate these to function.  
- To compare and contrast cell structures in eukaryotes and bacteria. |
| Theme 3: Metabolism | This theme outlines the key concepts of metabolism, the consumption and generation of energy by living cells. Lectures and practical classes on this theme should enable you:  
- To describe the essential differences between proteins, carbohydrates and lipids.  
- To describe the processes by which these molecules enter cells.  
- To comprehend the processes of generating energy for cellular function.  
- To compare and contrast energy generation in animals and plants. |
| Theme 4: Genetics | This theme introduces the key concepts of modern genetics, including what genes are, how they are regulated, how genetic information is transmitted and how modern molecular biology can use genetics to understand biology. Lectures and practical classes on this theme should enable you:  
- To describe the essential structures of genetic material (nucleic acids, genes, chromosomes).  
- To explain the processes by which cells divide.  
- To describe the principal steps in the control of gene expression and the production of functional proteins.  
- To relate these structures and processes to the inheritance of genetic characteristics.  
- To explain the uses of recombinant DNA technology in at least one situation relating to investigation of gene function. |
**Course Structure**

| Practical classes | Laboratory based experimentation is an important part of modern biology, and this course gives you the opportunity to conduct laboratory explorations and to acquire basic skills. The practical component of this course is designed as an exploration of cell structure and function, and of the genetic material of those cells. It is divided into three sequences of practicals that are linked to the lecture series:  
| o Course introduction and laboratory safety  
| o Exploring cell structure (Practicals 1-3)  
| o Exploring cell function (Practicals 4-6)  
| o Exploring genes (Practicals 7-9)  
| o Biological modelling (Practical 10) |
| There are goals for each individual practical class, and overall goals for each section. Each practical class is assessable. |
| **LABORATORY PREPARATION:** PRIOR to each class, you are expected to complete a pre-lab quiz in Moodle. Your demonstrator will discuss this requirement further with you in Week 1. |
| Your attendance at EVERY laboratory class is COMPULSORY, including the introductory session on laboratory safety and procedures in Week 1. Should you be unable to attend your practical class for any reason, you will not be able to do “make-up” labs. For unavoidable absences from practical classes, you must provide your demonstrator with a medical certificate or other professional documentation that supports the reason for your absence. See FAQ on page 5 and Expectations of Students on page 20 for details on absences from classes. |

| Lectures | Lectures serve to emphasise certain principles covered in the text, provide an overview, and connect the individual components of the course. The lectures also serve to update and extend text coverage, using examples from current research. All of the lecturers in Molecules, Cells and Genes are active in research and have well established reputations in the fields in which they teach. At UNSW, the people who teach you biology have made significant contributions to your area of study. |
| Unlike high school, we do not take roll at lectures, and there is no compulsion to attend. There are often ideas or concepts covered in lectures that may not necessarily be dealt with in your text. Lectures also serve to highlight areas that we believe you should focus on in the textbook. Please note that you are also expected to learn more than is introduced to you in the lectures. |
| We are aware that the students in this course have widely varying backgrounds in biology, so we are concerned that all students are being appropriately catered for. If you feel that a lecture is dealing with something that is already familiar to you, you may use it as an opportunity for revision, or choose not to attend. |
| We highly recommend that you attend all lectures, and that you listen and ask questions. There is no point in coming to a lecture to talk to your friends. Such chatting also makes it difficult for your fellow students who would like to listen, and for the person giving the lecture. Remember that it is a real person up there, not a TV screen, and that we can hear you as well as you can hear us. Repeated disruption of lectures by talking, or through other inappropriate behaviour, constitutes misconduct and can be punished according to University regulations. Lecturers may also ask a disruptive student to leave the lecture theatre. |
| Please note: Mobile phones are to be switched to silent during lectures and practicals. Lecture notes for all lectures will be made available on the BABS1201 Moodle site. Additionally, all lectures are recorded and are made accessible through the Echo360 link on the BABS1201 Moodle site. |
| Please be aware that whilst the lecture notes are comprehensive, they are no substitute for actually attending the lecture in person, and taking your own additional notes. |
## Graduate Attributes Developed in this Course

<table>
<thead>
<tr>
<th>Science Graduate Attributes</th>
<th>Activities / Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Research, inquiry and analytical thinking abilities</td>
<td>Guided laboratory practicals; independent and collaborative lab research (enzymes experiment); and independent research (biological modelling).</td>
</tr>
<tr>
<td>Capability and motivation for intellectual development</td>
<td>Small group discussions on critical thinking and the scientific method; independent research (biological modelling); Mastering biology online resources.</td>
</tr>
<tr>
<td>Ethical, social and professional understanding</td>
<td>Lectures address some of the ethical and social issues of biology, and the &quot;how to think like a scientist&quot; theme throughout the course addresses professional understanding.</td>
</tr>
<tr>
<td>Communication</td>
<td>Development of scientific writing skills through introduction to scientific literature (essay); written laboratory report; and oral presentation (biological modelling).</td>
</tr>
<tr>
<td>Teamwork, collaborative and management skills</td>
<td>Team independent research project (enzymes experiment); facilitation of group discussions in class and on Moodle; biological modelling team project.</td>
</tr>
<tr>
<td>Information literacy</td>
<td>Introduction to finding reviews and primary scientific literature (essay), independent research project (biological modelling).</td>
</tr>
</tbody>
</table>

### Teaching strategies

Lectures are used to introduce the concepts of fundamental cell biology and laboratory sessions are used to both complement the lecture material and provide practice in standard biological techniques used in research. Laboratories are used to encourage teamwork. Discussion groups and electronic resources referred to within scheduled laboratory classes are additionally designed to further reinforce the concepts presented in lectures and practised in the laboratory, and support students in their assigned projects.

The laboratory program forms an essential element of the students’ scientific training. The laboratory program, as integrated with the other components of the course, have been designed in accordance with the UNSW Guidelines on Learning that Inform Teaching (www.guidelinesonlearning.unsw.edu.au) to:

- Teach students the process of scientific inquiry through progressive cycles of critical analysis of their research and their own thinking;
- Facilitate multidisciplinary thinking to reflect current research and professional practice in the sciences;
- Reinforce deep learning and promote collaborative inquiry;
- Integrate students’ disciplinary understanding and research practice with the development of their communication skills, teamwork, and information literacy skills.
<table>
<thead>
<tr>
<th>What is BABS1201?</th>
<th><strong>Lecture topics</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Course introduction.</td>
</tr>
<tr>
<td></td>
<td>Use of course resources: lectures, Moodle, and practicals.</td>
</tr>
<tr>
<td></td>
<td>Assessments.</td>
</tr>
<tr>
<td></td>
<td>How to study and excel in your assessments.</td>
</tr>
<tr>
<td></td>
<td>Plagiarism.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Scientific literature</th>
<th><strong>Lecture topics</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Communication between scientists: literature, presentations, conferences.</td>
</tr>
<tr>
<td></td>
<td>Nature of peer review.</td>
</tr>
<tr>
<td></td>
<td>Primary literature and review articles.</td>
</tr>
<tr>
<td></td>
<td>Electronic databases.</td>
</tr>
<tr>
<td></td>
<td>Citations.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Learning outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>To be able to identify scholarly reports and to recognise the principles behind the style and content of scientific journals.</td>
</tr>
<tr>
<td>To understand how scientific arguments are built by reference to published scientific reports.</td>
</tr>
<tr>
<td>To appreciate the importance of verification in the scientific process.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Life</th>
<th><strong>Lecture topics</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>What is life?</td>
</tr>
<tr>
<td></td>
<td>Bacteria, archaea and eukaryotes.</td>
</tr>
<tr>
<td></td>
<td>Principal differences between bacteria and eukaryotic cells.</td>
</tr>
<tr>
<td></td>
<td>Evolutionary relationships between cell types.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Learning outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>To understand the characteristics of life.</td>
</tr>
<tr>
<td>To be able to identify the fundamental differences between bacteria and eukaryotic cells.</td>
</tr>
<tr>
<td>To comprehend that prokaryotes gave rise to single-celled organisms with a DNA-containing nucleus – the eukaryotes.</td>
</tr>
<tr>
<td>To understand the likely evolutionary relationships between the different domains of life.</td>
</tr>
<tr>
<td>To understand the processes of natural selection.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cells</th>
<th><strong>Lecture topics</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eukaryote organelle structure and function.</td>
</tr>
<tr>
<td></td>
<td>Bacterial cell structure.</td>
</tr>
<tr>
<td></td>
<td>The cytoskeleton.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Learning outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>To identify characteristic structures of eukaryotic and bacterial cells and to describe their basic functions.</td>
</tr>
<tr>
<td>To describe the concept of endosymbiosis.</td>
</tr>
<tr>
<td>To list the main components of the cytoskeleton and briefly describe their roles in the cell.</td>
</tr>
</tbody>
</table>
Please note that the lecture topics and learning outcomes listed below are a guide only. Individual lecturers may provide you with updated topics and learning outcomes.

<table>
<thead>
<tr>
<th>Macromolecules</th>
<th>Lecture topics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• The four main organic components of cells – nucleic acids, proteins, lipids, carbohydrates.</td>
</tr>
<tr>
<td></td>
<td>• Identification of important similarities &amp; differences in macromolecular structure.</td>
</tr>
<tr>
<td></td>
<td>• Introductory concepts of breakdown and synthesis.</td>
</tr>
<tr>
<td></td>
<td>• Functions of macromolecules – structural, food storage, enzymes.</td>
</tr>
<tr>
<td></td>
<td>Learning outcomes</td>
</tr>
<tr>
<td></td>
<td>• To identify characteristic structures of protein, carbohydrate and lipid molecules.</td>
</tr>
<tr>
<td></td>
<td>• To describe the principal elements of their formation from and breakdown to their molecular subunits.</td>
</tr>
<tr>
<td></td>
<td>• To identify the importance of these molecules in cell structure and in nutrition.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell Integrity</th>
<th>Lecture topics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Membrane structure.</td>
</tr>
<tr>
<td></td>
<td>• The fluid mosaic model.</td>
</tr>
<tr>
<td></td>
<td>• The roles of lipids and proteins in maintaining cell integrity.</td>
</tr>
<tr>
<td></td>
<td>• Membrane permeability, diffusion and osmosis.</td>
</tr>
<tr>
<td></td>
<td>Learning outcomes</td>
</tr>
<tr>
<td></td>
<td>• To comprehend the structure of the cell membranes and their function in maintaining cell integrity.</td>
</tr>
<tr>
<td></td>
<td>• To describe the different components of the cell membrane that play an important role in maintaining cell integrity.</td>
</tr>
<tr>
<td></td>
<td>• To explain the non-selective diffusion of some small molecules across cell membranes and osmosis.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cellular transport</th>
<th>Lecture topics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Transporter proteins.</td>
</tr>
<tr>
<td></td>
<td>• Passive and active transport.</td>
</tr>
<tr>
<td></td>
<td>• Ion transport and membrane potential.</td>
</tr>
<tr>
<td></td>
<td>• Vesicular transport.</td>
</tr>
<tr>
<td></td>
<td>Learning outcomes</td>
</tr>
<tr>
<td></td>
<td>• To explain the mechanisms by which small molecules may be selectively transported into and out of cells.</td>
</tr>
<tr>
<td></td>
<td>• To comprehend the concept of a membrane potential arising from ionic imbalances across cell membranes.</td>
</tr>
<tr>
<td></td>
<td>• To describe the different types of endocytosis.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Photosynthesis: Synthesising food from energy</th>
<th>Lecture topics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Overview of photosynthesis.</td>
</tr>
<tr>
<td></td>
<td>• Light harvesting.</td>
</tr>
<tr>
<td></td>
<td>• The light reactions of photosynthesis.</td>
</tr>
<tr>
<td></td>
<td>• The Calvin cycle.</td>
</tr>
<tr>
<td></td>
<td>Learning outcomes</td>
</tr>
<tr>
<td></td>
<td>• To comprehend the functions of the different stages in photosynthesis - light harvesting, the conversion of light energy into chemical energy and carbon dioxide fixation.</td>
</tr>
<tr>
<td></td>
<td>• To comprehend the overall organisation of the light reactions in photosystems I and II.</td>
</tr>
<tr>
<td></td>
<td>• To describe, in overview, the fixation of carbon dioxide and synthesis of glucose in the Calvin cycle.</td>
</tr>
<tr>
<td></td>
<td>• To compare and contrast the generation of energy from photosynthesis or oxidation.</td>
</tr>
</tbody>
</table>
Please note that the lecture topics and learning outcomes listed below are a guide only. Individual lecturers may provide you with updated topics and learning outcomes.

<table>
<thead>
<tr>
<th>Metabolism I: Metabolic concepts</th>
<th>Lecture topics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Catabolism and anabolism.</td>
</tr>
<tr>
<td></td>
<td>• The role of ATP in “energy” metabolism.</td>
</tr>
<tr>
<td></td>
<td>• Nutritional and metabolic diversity.</td>
</tr>
<tr>
<td></td>
<td>• Respiration and fermentation.</td>
</tr>
<tr>
<td></td>
<td>• Metabolic control.</td>
</tr>
<tr>
<td>Learning outcomes</td>
<td>• Explain the differences between anabolism and catabolism.</td>
</tr>
<tr>
<td></td>
<td>• Describe the process of cells breaking down molecules to release chemical energy, render them harmless or allow for recycling.</td>
</tr>
<tr>
<td></td>
<td>• Explain the basic model for enzyme catalysis.</td>
</tr>
<tr>
<td></td>
<td>• Describe the structure and function of ATP.</td>
</tr>
<tr>
<td></td>
<td>• Explain the four main nutritional modes utilised by organisms.</td>
</tr>
<tr>
<td></td>
<td>• To comprehend the importance of oxygen and respiration in higher animals and plants.</td>
</tr>
<tr>
<td></td>
<td>• Explain the concept of metabolic control via feedback inhibition.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Metabolism II: Extracting energy from food</th>
<th>Lecture topics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Overview of the catabolism of carbohydrate, fat and protein.</td>
</tr>
<tr>
<td></td>
<td>• Overview of glycolysis, the TCA cycle and the respiratory chain.</td>
</tr>
<tr>
<td></td>
<td>• Chemiosmosis - the formation of ATP by oxidative phosphorylation.</td>
</tr>
<tr>
<td></td>
<td>• ATP yields from glucose catabolism.</td>
</tr>
<tr>
<td>Learning outcomes</td>
<td>• Describe the convergent catabolism of different macromolecules.</td>
</tr>
<tr>
<td></td>
<td>• Describe the central features of glycolysis, the TCA cycle and oxidative phosphorylation.</td>
</tr>
<tr>
<td></td>
<td>• To comprehend the basic principles of chemiosmosis - the generation of a proton gradient by, for example, the respiratory chain and the utilisation of the gradient by ATP synthase.</td>
</tr>
<tr>
<td></td>
<td>• To explain the advantages of respiration over fermentation with respect to energy yields.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>From gene to function</th>
<th>Lecture topics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• What is the genetic information?</td>
</tr>
<tr>
<td></td>
<td>• An introduction to nucleic acid structure.</td>
</tr>
<tr>
<td></td>
<td>• Bases as code.</td>
</tr>
<tr>
<td>Learning outcomes</td>
<td>• Describe the basic structure of nucleic acids.</td>
</tr>
<tr>
<td></td>
<td>• Explain how genetic information is encoded in nucleic acids.</td>
</tr>
<tr>
<td></td>
<td>• Identify the differences between DNA &amp; RNA.</td>
</tr>
<tr>
<td>Topic</td>
<td>Lecture topics</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>DNA replication</td>
<td>• Mechanisms of DNA synthesis in bacteria and eukaryotes.</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell division and reproduction</td>
<td>• Nature of genes and chromosomes.</td>
</tr>
<tr>
<td></td>
<td>• Cell division: mitosis and meiosis.</td>
</tr>
<tr>
<td>Gene expression I: Transcription</td>
<td>• DNA(\rightarrow)RNA(\rightarrow)protein.</td>
</tr>
<tr>
<td></td>
<td>• The genetic code.</td>
</tr>
<tr>
<td></td>
<td>• Transcription: The synthesis of RNA from a DNA template.</td>
</tr>
<tr>
<td></td>
<td>• Differences in gene expression between bacteria &amp; eukaryotes.</td>
</tr>
<tr>
<td>Gene expression II: Translation</td>
<td>• Overview: the translation of mRNA into amino acids.</td>
</tr>
<tr>
<td></td>
<td>• Transfer RNA and its role in translation.</td>
</tr>
<tr>
<td></td>
<td>• The ribosome as the protein synthesis factory.</td>
</tr>
<tr>
<td></td>
<td>• The three stages of translation.</td>
</tr>
<tr>
<td>The polymerase chain reaction</td>
<td>• Polymerase chain reaction (PCR).</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Topic</td>
<td>Lecture topics</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Mutation</td>
<td>Errors in reproduction. Environmental influences (radiation, chemicals, viruses). “Beneficial mutations” and natural selection. Loss of function or changed function. Gene duplication. Relationship to selected disease. Mutations in the immune system.</td>
</tr>
<tr>
<td>Mendel's laws of heredity</td>
<td>Mendel’s laws. Essential concepts in genetics: allele vs. locus, genotype vs. phenotype, homozygosity vs. heterozygosity, recessive vs. dominant</td>
</tr>
<tr>
<td>Mechanisms of inheritance</td>
<td>Modes of inheritance (single-locus, Mendelian traits). Inheritance of complex traits. Vertical inheritance vs. horizontal gene transfer.</td>
</tr>
<tr>
<td>Population genetics</td>
<td>Hardy-Weinberg law. Evolutionary forces that change allele and genotype proportions.</td>
</tr>
<tr>
<td>Course review</td>
<td>Overview of BABS1201. Exam structure and tips. Student questions.</td>
</tr>
</tbody>
</table>
## Administrative Matters

### Expectations of Students

A pass in BABS1201 is conditional upon a satisfactory performance in both the assessment and practical programs. We expect that you will have:

- Attempted/submitted all assessment items.
- Attended all of the practical classes (an attendance record is kept).
- Kept an accurate and up-to-date laboratory manual, including the recording of all data and completion of calculations and questions.

**PLEASE NOTE** that if students attend less than 80% of their possible classes they may be refused final assessment. Your attendance at classes will be monitored. Holidays (local or international) are NOT considered a valid reason for student absences from classes and assessments. For more details on UNSW class attendance policies, please refer to: [https://student.unsw.edu.au/attendance](https://student.unsw.edu.au/attendance)

### Assignment Submissions

Requirements vary with each assigned task. All information regarding submissions is explained in this manual, by your practical class demonstrator and online via Moodle announcements.

### Equity and Diversity

Those students who have a disability that requires some adjustment in their teaching or learning environment are encouraged to discuss their study needs with the course convenor prior to, or at the commencement of, their course, or with the Equity Officer (Disability) in the Equity and Diversity Unit (93854734 or [http://www.studentequity.unsw.edu.au/](http://www.studentequity.unsw.edu.au/)).

Issues may include access to materials, signers or note-takers, the provision of services and additional exam and assessment arrangements. Early notification is essential to enable any necessary adjustments. Information on designing courses and course outlines that take into account the needs of students with disabilities can be found on the above website.

### Student Complaint Procedure

| BABS School Contact | Marc Wilkins  
| m.wilkins@unsw.edu.au |  
| Tel: 9385 3633 |

| Science Faculty Contact | Gavin Edwards  
| Associate Dean (Undergraduate programs) | g.edwards@unsw.edu.au |
| Tel: 9385 7111 |

| University Contact | Student Conduct and Appeals Officer (SCAO) within the Office of the Pro-Vice-Chancellor (Students) Registrar |
| Tel: 9385 8515 | studentcomplaints@unsw.edu.au |
## Special Consideration and Further Assessment

### Explanation

Students who believe that their performance, either during the session or in the end of session exams, may have been affected by illness or other circumstances may apply for special consideration. For BABS1201, applications can be made for in-session assessments tasks and the final examination.

**Students must make a formal application for Special Consideration for the course/s affected as soon as practicable after the problem occurs and within three working days of the assessment to which it refers.**

Students should consult the “Special Consideration” section of Moodle for specific instructions related to each BABS course they are studying. Further general information on special consideration can also be found at [https://student.unsw.edu.au/special-consideration](https://student.unsw.edu.au/special-consideration).

### How to apply for special consideration

Applications must be made via Online Services in myUNSW. **You must obtain and attach Third Party documentation before submitting the application.** Failure to do so will result in the application being rejected. Log into myUNSW and go to **My Student Profile tab > My Student Services channel > Online Services > Special Consideration**. After applying online, students must also verify their supporting documentation by submitting to UNSW Student Central:

- Originals or certified copies of your supporting documentation, and
- A completed [Professional Authority form](https://my.unsw.edu.au/student/academiclife/ProfessionalAuthority.pdf).

The supporting documentation must be submitted to Student Central for verification within three working days of the assessment or the period covered by the supporting documentation. Applications which are not verified will be rejected.

Further details are also available:

[https://my.unsw.edu.au/student/academiclife/ProfessionalAuthority.pdf](https://my.unsw.edu.au/student/academiclife/ProfessionalAuthority.pdf)

Students will be contacted via the online special consideration system as to the outcome of their application. Students will be notified via their official university email once an outcome has been recorded.

### Supplementary examinations

The University does not give deferred examinations. However, further assessment exams may be given to those students who were absent from the final exams through illness or misadventure. Special Consideration applications for final examinations will only be considered after the final examination period when lists of students sitting supplementary exams/tests for each course are determined at School Assessment Review Group Meetings. Students will be notified via the online special consideration system as to the outcome of their application.

**It is the responsibility of all students to regularly consult their official student email accounts and myUNSW in order to ascertain whether or not they have been granted further assessment.**

In Session 1, the BABS1201 Supplementary Examination is scheduled on:

**Friday 14 July**

Further assessment exams will be offered on this day only and failure to sit for the appropriate exam may result in an overall failure for the course. Further assessment will **NOT** be offered on any alternative dates.
### Academic Honesty and Plagiarism

Plagiarism is the presentation of the thoughts or work of another as one’s own.

Examples include:

- Direct duplication of the thoughts or work of another, including by copying work, or knowingly permitting it to be copied. This includes copying material, ideas or concepts from a book, article, report or other written document (whether published or unpublished), composition, artwork, design, drawing, circuitry, computer program or software, website, Internet, other electronic resource, or another person’s assignment without appropriate acknowledgement.
- Paraphrasing another person’s work with very minor changes keeping the meaning, form and/or progression of ideas of the original.
- Piecing together sections of the work of others into a new whole.
- Presenting an assessment item as independent work when it has been produced in whole or part in collusion with other people, for example, another student or a tutor.
- Claiming credit for a proportion a work contributed to a group assessment item that is greater than that actually contributed.
- Submitting an assessment item that has already been submitted for academic credit elsewhere may also be considered plagiarism.
- The inclusion of the thoughts or work of another with attribution appropriate to the academic discipline does not amount to plagiarism.

Students are reminded of their Rights and Responsibilities in respect of plagiarism, as set out in the University Undergraduate and Postgraduate Handbooks, and are encouraged to seek advice from academic staff whenever necessary to ensure they avoid plagiarism in all its forms.

The Learning Centre website is the central University online resource for staff and student information on plagiarism and academic honesty. It can be located at: www.lc.unsw.edu.au/plagiarism

The Learning Centre also provides substantial educational written materials, workshops, and tutorials to aid students, for example, in:

- Correct referencing practices.
- Paraphrasing, summarising, essay writing, and time management.
- Appropriate use of, and attribution for, a range of materials including text, images, formulae and concepts.

Individual assistance is available on request from The Learning Centre.

Students are also reminded that careful time management is an important part of study and one of the identified causes of plagiarism is poor time management. Students should allow sufficient time for research, drafting, and the proper referencing of sources in preparing all assessment items.
A. Online Laboratory Safety Quiz – COMPULSORY

In order to be permitted to take part in laboratory classes from Week 2 onwards, you must complete an online Laboratory Safety Quiz that is accessed through the BABS1201 Moodle site. Prior to your Week 2 laboratory class, go to the BABS1201 Moodle site and enter the ‘Laboratory Safety Quiz’ module by clicking on the appropriate icon on the home page. Follow the instructions provided there and use the above information on occupational health and safety that you have discussed with your demonstrator today to complete the quiz. When you have finished the quiz and submitted all your answers, you will receive a mark out of 12. If ANY of your answers were incorrect, then you MUST attempt the quiz again and keep repeating this process until you have scored a mark of 12/12 (that is, 100%). Since it is crucial that you are aware of all important health and safety rules and regulations, you can attempt the quiz as many times as required for you to get ALL the answers correct. Your final quiz mark will be checked prior to your lab in Week 2.

This quiz is compulsory, but does not contribute to your grade.

If you have not scored 100% in the quiz by 9am on the day of your Week 2 practical class you will NOT be permitted to attend that lab class or any subsequent lab class until you have satisfied this requirement.
B. Session Test 1 – 15%

Week 4, Tuesday 22 or Wednesday 23 March, during your enrolled lecture time

This 40 minute test covers ALL theory and practical material from Weeks 1 to 3 (inclusive) of session. The format of the exam is multiple choice questions.

The exam will be conducted in your enrolled second lecture time, Wednesday or Friday, and venue in Week 4. Due to seating restrictions, it is essential that you attend the lecture time in which you are enrolled. Students who complete this test during the wrong lecture time will receive zero marks for this assessment item.

More information about this test will be provided in lectures and via Moodle. If you miss this test due to illness or misadventure, please apply for special consideration online (see page 19 for instructions).
C. Scientific Literature Essay – 10%

Your first BABS1201 assignment is a reflective essay task that compares a scientific review, on a biological topic of your choice, with a primary research article. Ensure you attend the Week 3 lecture on Scientific Literature for help with this.

**Essay DUE** in your lab class (hard copy) in Week 5, with an electronic copy submitted to Turnitin via Moodle.

Your essay must be handed in during your Week 5 lab class and submitted electronically on the BABS1201 Moodle site. Make sure that you read all instructions below carefully and pay close attention to information presented in lectures and on Moodle for any updates.

This assignment is designed as a first university writing assignment. It will be followed by other assignments in this course, and in other courses, that will progressively introduce you to the demands of scientific report writing. This assignment, however, is not an exercise in scientific report writing. It is a more personal piece of writing which in part reflects upon your own experience locating and reading a scientific article.

The objectives of the task **do not focus on an understanding of the scientific literature**. A major objective is to see if you can write clearly and concisely. If, as a result of this assessment, we think you have problems writing, we will refer you to the **UNSW Learning Centre** for assistance.

The essay should be approximately 1000 words long. You must perform a word count and indicate the count at the beginning of your essay. You will not be penalised for going slightly over 1000 words, but penalties will apply to essays that exceed the limit by more than 10%.

From information presented in your BABS1201 lectures and lab discussions, you should become familiar with the different types of peer-reviewed literature used by the scientific community. **One of the major objectives of this assignment is to ascertain whether YOU are able to differentiate between primary and secondary (review) journal articles,** so your demonstrator or lecturers will **NOT** provide you with an answer if you ask them to check the articles you are considering. They will, however, discuss **HOW** to differentiate between the two in class.

**ESSAY INSTRUCTIONS**

Your essay task is to find a **REVIEW** article from any biological science discipline, and then from the cited literature that is mentioned in the review, select one **PRIMARY** research article. Your essay must include and will be assessed on the following criteria:

1. **A simple description of the nature of the review article.** This should include a description of the topic that is being reviewed as well as a description of the audience for which the review has been written. This will require you to reflect upon the journal, and the audience that the journal is typically written for.

2. **A description of the discipline that you think best describes the research that is reported in the article.** For example, is it zoology, microbiology, genetics or something else that you think best describes the work? Remember that there may be more than one discipline name that you can think of, and you could discuss the different possibilities if that is the case. You should say a little bit about what you think the discipline is about. Of course you do not have space to do this in any detail. In some cases it may be easy. In others, the boundaries of the discipline may be unclear to you. Feel free to discuss your uncertainties. Remember that this is a **reflective** essay.
3. **A description of the journal.** Write about the relationship between the journal you have chosen and the discipline. In some cases, this will mean you will find out a little about a professional society that is associated with the journal. The journal may be a general one, which 'serves' many disciplines, or may be a journal that is strongly associated with a particular scientific sub-discipline. The journal may target regional, national or international audiences.

4. **A description of the purpose of the review and a brief summary of the results of the investigations that are reported within it.** Don't get bogged down with the detail, focus on the main points only. You may not understand very much and that is okay – you are a first year biology student and the article may be written for an audience of specialised researchers. If this is the case, do not panic! You will not be penalised as long as you can write a sensible reflection eg. what was interesting? what did you learn? what was difficult to understand? etc. (Remember this is a reflective essay, where you are free to discuss both your success and difficulties!) This part of your essay could look something like this: “I chose this article as I am interested in Australian animals, and this paper was about the behaviour of wombats. However, it much of it was difficult to understand. The page included 15 words that I had never seen before. Although I used a dictionary to clarify the meaning of most of the words, I was still unable to make any sense of the article as I had no general knowledge of animal behaviours, such as foraging and being territorial, that this article built on etc.”

5. **A brief description of the references that are cited in the review article.** For example, you may talk about the number of journals; you could try to judge which citations are from the primary literature and which are from the secondary literature (review articles). Do the referenced publications all appear to be from peer-reviewed journals, or are there other kinds of publications that are cited? Are the references all very recent publications, or are there some older works? Can you get a sense of the kinds of journals that are being cited? Please note – some review articles refer to hundreds of journal articles. So do not analyse exhaustively the entire reference list! In this section we want you to give us your brief general impressions.

6. **The title, complete reference details and brief description of one PRIMARY research paper from the reference list of your review article.** Locate and read through a primary research article selected from the citations in your review article. Briefly describe the article in your essay and discuss its role in or contribution to the review article.

7. **A sentence or two on your understanding of the nature and purpose of primary literature compared to secondary literature.**

You must carefully read the above points and address them. Omitting to include one of the criteria will result in a loss of marks. The clarity of your writing is also important, so ensure you leave sufficient time to proof read your work. Remember that you can score full marks without understanding the journal articles if you just follow the instructions above.

Please note that you MUST provide full reference details of both the review article and primary research article (and any other articles you use) at the end of your essay so markers can assess your essay accurately. This does not mean you include the references cited within the two articles ie. do NOT paste in the references section from your two articles, only provides details of references you use.

Write as if your reader is another BABS1201 student, this means that your descriptions of the science need to be in your own words. All assignments will be screen for plagiarism, so it is particularly important.
D. Team Enzyme Project – 15%

This task is a course assessment component and is worth 15% of your final mark in BABS1201. In order to complete this task, you will be assigned to a smaller team of two students within your demonstrator group.

Marks will be allocated as follows:

There are three assessable components to this project. Depending upon the item, the grading may be as a team (pair) or individual.

- Experimental notes (2%) (individual submission – both students must record the results)
- Final report (10%) (individual submission)

Objectives:

- To perform background research on enzyme structure and function.
- To design an experimental protocol to explore the activity of one of the following enzymes:
  - Catalase
  - Bromelain
  - Rennin
- To carry out and collect data from your group-devised experimental procedures.
- To construct graphs or tables of these results that would be suitable for inclusion in a formal scientific report.
- To compile a scientific report that accurately conveys your group’s experimental findings.

Project Background:

Enzyme activity is influenced by factors such as concentration of the enzyme, concentration of the substrate, pH and temperature. All enzymes are affected in specific ways by these factors. Thus, there are optimal catalytic conditions for each enzyme. Since enzymes are found in living organisms, the optimal pH and temperature reflect the environmental conditions in which these organisms function. For example, in mammals the pH of cell cytoplasm is approximately 7, but the pH of the stomach cavity can be as low as 2-3. Therefore, enzymes that function within mammalian cells typically have a neutral pH optimum, whereas the stomach enzyme pepsin works best at an acidic pH. Similarly, the optimal temperature for an enzyme reflects the temperature of its natural environment (~37°C in the case of human enzymes).

As a small group of two students, you are required to design and implement an experimental protocol that investigates factors that affect enzyme activity (as described above and in your ‘Macromolecules’ lecture). This team project will require your participation over several weeks of the session. The detailed schedule of the different components of this project are presented in the table on the following page.
# Weekly Schedule for the Team Enzyme Project:

<table>
<thead>
<tr>
<th>When?</th>
<th>Component</th>
<th>Description of Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 3</td>
<td>Discussion on Experimental Design</td>
<td>You will be allocated your team partner and assigned a specific enzyme to study. You will reflect upon the fundamental aspects of good scientific experimental design. You will also have the opportunity to discuss the requirements of the group experimental design project.</td>
</tr>
<tr>
<td>Weeks 4-5</td>
<td>Design of Experimental Protocol</td>
<td>You will meet with your team partner to design your enzymes experiment and construct a detailed protocol that outlines the aims, materials required, methods to be performed by EACH member and the expected outcomes (see pages 27-34). Also design a template you will use to record your results on the day.</td>
</tr>
<tr>
<td>Week 7</td>
<td>Experimental Protocol Submission</td>
<td>During your lab this week, you will submit your final draft experimental protocol to your demonstrator for review. Your team’s protocol must include enzyme background information, the aims and purpose of the experiment, a complete list of the materials and equipment required, a detailed description of the experimental methods/procedures to be employed by EACH team member, and the expected outcomes of the experiment. Your demonstrator will give you feedback relating to the feasibility of your protocol, along with any suggestions for improvement. Include a template you will use to record your results on the day. <strong>FINAL protocols must be submitted no later than WEEK 7.</strong> If your group does not submit their protocol on time, you will have marks deducted from your final report and may not have the necessary reagents and equipment available to you in WEEK 9.</td>
</tr>
<tr>
<td>Week 9</td>
<td>Enzymes Practical</td>
<td>During Practical 6, you will carry out the procedures outlined in your final experimental protocol. You must record all of the data obtained in this class, as you will be required to submit these notes to your demonstrator with your report. <strong>Each member MUST record the team’s data and observations made on the day.</strong></td>
</tr>
<tr>
<td>Week 10</td>
<td>Report Submission</td>
<td>Lastly, you will work <em>individually</em> outside class time to compile a scientific report in which your team’s experimental findings are to be communicated. Your report must be written in scientific format with the following section headings: Introduction, Aims, Materials and Methods, Results, and Discussion. Your discussion should also include reflective comments on the entire group project experience. Such comments could describe the challenges you faced, whether you obtained your expected outcomes (and if not, why?), the valuable insights you gained into experimental design and/or enzyme properties, as well as any suggestions for future experiments. Your report should be no longer than 6 pages (inclusive of figures, tables, graphs etc). Scientific report guidelines and assessment requirements can be found on pages 27-34 of this manual. You are to submit your complete written report to your demonstrator at the commencement of your lab class in Week 10 and a copy online. <strong>This report and your overall contributions to the group project are worth 15% of your final assessment mark in BABS1201.</strong></td>
</tr>
</tbody>
</table>
Experimental Design Task:

- In Week 3, you will be assigned to a team of two students for the Team Enzymes Project. Your team will also be assigned a specific enzyme.

- Once assigned to your team, you are required to meet with your Enzymes Project partner in your own time to design an experimental protocol that investigates enzyme structure and/or function.

- You will learn about enzymes and some of the factors that can significantly affect enzyme activity in your BABS1201 lectures. For example, within your laboratory environment, it is quite simple to test the effect of temperature and/or pH changes on the activity of various enzymes.

- Your team will be allocated an enzyme that is readily obtainable and relatively easy to study within the lab. But YOU will be required to supply the enzyme source. Remember, the source of your enzyme should be something that is readily available from a supermarket etc., and NOT from a commercial scientific enzyme supplier! Developing ideas for your experimental protocol should not be difficult either. If you perform some general internet searches for simple experiments with enzymes, you should find many examples that you can use as the stimulus for your own experimental design. Just remember to reference any protocols that you adopt for this project in your final scientific report.

- To facilitate and allow monitoring of both individual and team contributions to this project, the experimental design task has been divided into two main parts whereby Part 1 requires INDIVIDUAL contributions from each team member and Part 2 requires a TEAM effort for its completion. See descriptions below (and please note that your demonstrator will provide you with further guidance on the content and length of your protocol):

  PART 1  
  A) Each team member should conduct their own individual research and provide a brief background on their enzyme (including its source, structure, substrate and cofactor requirements etc.);
  B) Each team member should also provide their own explanation of the experimental hypothesis that their team is going to be testing.

  PART 2  
  A) Together, both team members should provide a list of all equipment and reagents required, as well as a basic step-by-step description of the experimental procedures to be carried out. This should include a clear indication of the unique roles that each team member will play during the experiment;
  B) Together, both team members should also provide labelled schematic representations or diagrams of the procedures to be carried out and apparatus to be employed;
  C) Together, both team members should provide a completed risk assessment for their experiment. Information on risk assessments and how to construct them can be found in the ‘Team Enzyme Project’ section in Moodle.

PLEASE NOTE that a ‘template’ file for your team’s experimental protocol will be available for you to use in Moodle (go to the ‘Team Enzyme Project’ section). This file will contain space for you to provide all the information requested above. You will be required to submit the completed file to your demonstrator in Week 7 (Practical 5) and your approved protocol will then need to be submitted online via Moodle for use by technical staff. Updated information for submitting your team protocol may be provided in lectures and Moodle.
Additional Notes:

- Just remember that your experimental protocol should be simple and relatively brief! You are required to complete the whole experiment within 3 hours (including any repetitions of the procedure – for accuracy and reproducibility!). So make sure that your experiment does not require any complicated set-up procedures or very long incubation periods, for example. And remember, quality is better than quantity; plan your experiment carefully so that you don’t need to rush it. But also make sure that there is enough work planned so that both you and your team partner have separate tasks to carry out during the experiment.

- Most equipment and reagents that you require to conduct your experiment will be supplied by our technical staff. Below, you will find a list of the items that can be made available to you on the day of your experiment, but only if you specifically request them in your protocol. If you require items that are NOT on the lists below, you can ask your demonstrator to find out if the item(s) can be provided by the technical staff. But if this is not possible, then you may need to find and bring the items yourself or re-design your protocol.

Resources:

Below, you will find a list of equipment, reagents and solutions that our technical staff will be able to provide upon your team’s request. You should aim to design your experiment based on this list. Please note, however, that the quantity of each item or solution may be limited in some cases.

Equipment available:

- Test tubes
- Delivery tubes with rubber stoppers
- Beakers
- Measuring cylinders
- Glass stirring rods
- Mortar and pestle
- Watch glasses
- Volumetric flasks
- Conical flasks with rubber hoses
- Thermometers
- Timers
- Scalpels
- Test tube racks
- Water baths
- Stop watches
- Rubber bands
- Metal spoons
- Petri dishes
- Balance
- Razor blades
- Plastic ‘squish-bulb’ transfer pipettes

Reagents and solutions available:

- Universal indicator
- HCl (0.1 – 2.0%)
- 1M HCl (stock solution)
- NaCl
- 3% hydrogen peroxide solution
- 1M NaOH
- Buffer solutions
- Benedict’s solution
- Iodine (0.002M)
- Phenolphthalein
- Starch solution (0.75mg/ml)
- Cheese cloth
- Hot water baths (35°C, 45°C, 50°C, 60° and 90-100°C)
- Crushed ice

PLEASE NOTE that many of the above resources are limited in supply. To ensure that your experimental needs are provided on the day of your class, you must clearly specify the number of each piece of equipment required and also the approximate volume/amount of each reagent required in your experimental protocol. And you must also make sure that you submit your approved protocol online as instructed above.
STRUCTURING A SCIENTIFIC REPORT:

One of the skills you need for this course is the ability to write a clear, concise and well-referenced report of your experimental results and how you achieved them. While there are specific requirements for a scientific report, the ability to write a clear report will be of benefit to you whatever career you undertake.

Below are some general guidelines for constructing the different sections of a typical scientific report.

Introduction and Aims:

Your aim should be based on your hypothesis, and tell your reader what it is that you are trying to achieve with your experiment. You should also provide an introduction to your reader that tells them what other researchers have done in this area of work (citing references of course). Never assume that your reader knows the work as well as you do!

Methods:

When you design an experiment, you need to be sure of two things:

- The method you use will work.
- The methods you use will give you a result that means something.

The essence of a good scientific finding is that you or other people can get the same result when they do the same experiment, i.e. it is reproducible. Because very small differences in the way you do an experiment can affect your results, you must provide your reader with enough information about your methods that they can do exactly the same experiment. This can include telling them where you bought reagents, at exactly what temperature and for what times you carried out procedures, and even what machines you used.

Results:

In this section, you should identify what you found in your experiment. You need to show your exact data (not an approximation) and this can be in the form of a graph or a table: make it as easy as possible for your reader to understand what you have done and what the result is. You need to label your table or graph to make it even clearer. You should also describe in words your experiment and what its result was.

For example, the following table shows the result of an experiment to determine whether six different cells have a nucleus and/or organelles.

<table>
<thead>
<tr>
<th>Cell origin</th>
<th>Nucleus</th>
<th>Mitochondria</th>
<th>Chloroplasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human cheek</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Bacterial culture</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Onion smear</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Lettuce leaf</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Raw steak</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
</tr>
</tbody>
</table>

You also need to explain this to your reader.

e.g. "A range of different cell types were stained as described in the methods, and analysed by light microscopy for the presence of nuclei, mitochondria and chloroplasts. The results show a distribution of different organelles depending on the source of the cells".

Of course, most data can be presented in more than one way.
Discussion and conclusions:
The final part of your report is the discussion and analysis of your results, and the conclusions or meaning you can draw from them.

You also in this section can say how your results compare with those of other researchers.

References:
In this section you list all the other research papers or books that you have used as background material or references in your Introduction/aims, methods, results and conclusion.

TEAM ENZYME PROJECT REPORT GUIDELINES AND MARKING SCHEME:

Working individually, you must now compile a scientific report that is to be written and presented in a style similar to that of a primary scientific research article. In this report, you must describe the background details of your allocated enzyme and the hypothesis that you were testing, along with a thorough explanation of the materials and methods that you used, the experimental method employed, your experimental results, and a discussion of the experimental outcomes and observations.

The discussion section of your report must also contain reflective comments that describe your own personal contributions to the project and your thoughts on the overall project experience (for example, what you liked or didn’t like; what you found challenging or easy, etc.). See below for a detailed description of the report structure and requirements.

Each Team member is to submit their own individual report to their demonstrator in Week 10. Much your protocol submission can be used in the first parts of your report, while the results and discussion sections will need to be written up individually after the experiment. Please note that reports are to be written on an individual basis. Any cases where there are significant similarities between student reports in the report sections that should have been prepared individually (i.e. the Introduction, Results and Discussion sections) may be questioned for plagiarism. Also note that in cases where it is clearly evident that an individual team member did not make a satisfactory effort to contribute equally to the project, a reduction in marks may be applied to the final report mark of the team member in question.

Your report is to be submitted as a hard copy to your demonstrator, and should include a cover page with all the necessary details completed. Assignment cover sheets can be downloaded from the “Assessments” section in Moodle.

Enzymes Report DUE DATE:

Your report is due at the BEGINNING of your lab class (Week 10 practical time slot and venue) and is worth 15% of your final assessment mark in BABS1201. Your enzyme protocol submission forms part of your final report (eg. the methods section) along with the experimental activities. The penalty for late reports (including those handed in after the lab has started) is 10% per day, including weekends.

Report structure:

Below are some guidelines for the structure and content of your report, including an approximate distribution of marks for each section. Approximate page lengths are also provided for each section, but please note that you will not be penalised for exceeding these where it is necessary to do so.

Introduction (3 marks) - Approximately 0.5 to 1 page in length

The Introduction should include:

- A brief and general description or definition of enzymes and enzyme function.
- A more detailed description of the specific enzyme you examined in your experiment.
- A clear hypothesis and aim(s) of your experiment, with reference to the type of results you expected to obtain and what they might tell you about enzyme structure and/or function.
Materials and methods (1.5 marks) - Approximately 1 page in length (not including diagrams)

This section should be similar to the protocol you have already submitted to your demonstrator, except that it should include any changes or modifications to your original experimental plan. Your Materials and Methods section should include:

- A concise list of all reagents and equipment used in your experiment.
- A complete description of the experimental procedure in the form of a list of all the experimental steps performed by your group – as performed on the day (that is, include any deviations to your original protocol). Include sufficient detail that another BABS1201 student could replicate your experiment.
- Optional: you may include diagrams to depict one or more of the steps in your experimental procedure.
- If you have adapted your protocol from another source, you must cite the reference to this here.

Results (2 marks) - Approximately 1 to 2 pages in length

The Results section should include:

- A brief description of the results, including any additional observations that were made on the day.
- Tables and/or graphs of the data your group collected during your experiment.
- REMEMBER: the purpose of the results section is to accurately and effectively communicate your results to the reader, and not to attempt to interpret them specifically – that is instead the purpose of the “Discussion” section of your report.
- HINTS: A reader should be able to understand your results by reading the text alone (if the figures/tables were removed). Do not start the Results section with a figure or table, look at a scientific paper or a textbook, you need to explain a concept and use the figures and table to enhance the message.

Discussion (6 marks) - Approximately 2 to 3 pages in length

The Discussion section should include:

- A more thorough description of your results with respect to the aim(s) of your experiment. Did you expect the results you obtained and observations that you made? If not, why do you think your experiment did not proceed as expected? What were the major sources of possible error in your experiment?
- An answer to the following question: what is the significance of the results? what do your results/observations tell you about enzyme structure and/or function?
- Comments on how your experiment might be improved if it was repeated.
- ENSURE YOU ALSO INCLUDE: Reflective comments on the experience you gained in designing and then implementing your own experimental protocol (for example: the difficulties that were faced, the valuable insights that were gained, etc.). In this section, you should also provide a very clear description of your specific contributions to the overall project, including any comments on how you might work differently in any future team projects (if applicable). This is not usually included in a scientific report, but is assessed as it shows evidence of your learning process.

References (0.5 mark)

The References section should include:

- A complete list of any internet sites, textbooks or other references that you used to design your experimental protocol.
- Any other references such as journal articles or text books that you may have obtained information from for the purpose of writing your introduction, discussion or any other part of your report.
- Only include references you have cited in-text
- We recommend that you use the APA referencing style (or something very similar to this) for your reference list and internal citations.
Report Checklist:

☐ Report is in correct format, with an Introduction, Material and Methods, Results, Discussion and References.

☐ Any material used from other sources is cited in the text, and given in correct format in the References.

☐ The reference list should be only of references cited in the text.

☐ Both tables and figures should be included in the results section of the report.

☐ Tables, graphs and drawings are given a title and legend, and are clear and unambiguous.

☐ Attach your experimental notes from Week 9.

☐ Printed Group Assignment cover sheet is attached to the front of your report.

E. Session Test 2 – 15%

**Week 11, Monday or Tuesday, during your enrolled lecture time**

This 40 minute test covers ALL theory and practical material from Weeks 4 to 10 (inclusive). The format of the exam is multiple choice questions.

The exam will be conducted in your enrolled first lecture time, Monday or Tuesday, and venue in Week 11. Due to seating restrictions, it is essential that you attend the lecture time in which you are enrolled. Students who complete this test during the wrong lecture time will receive zero marks for this assessment item.

More information about this test will be provided in lectures and via Moodle. If you miss this test due to illness or misadventure, please apply for special consideration online (see page 19 for instructions).

F. Biological modelling – 10%

**Week 13, during your enrolled practical time**

For this assessment you will create a model of a biological system and present / display it in your practical class. Documenting your planning and progress as a team will also form part of this assessment and will be handed in during class.

In order to complete this task, you will be assigned to a small group of four students within your demonstrator group. Together, you need to chose a major biological system or concept that you will explain in class by presenting and demonstrating a model. You will need to meet both in and outside class time to design and construct your model. The attendance, roles, discussion, allocation of duties etc of these meetings must be documented by completing a downloadable template from Moodle. Your notes also need to document communications made via email / online discussions.
Suggested timeline for the Biological Modelling project:

<table>
<thead>
<tr>
<th>When</th>
<th>Component</th>
<th>Description</th>
</tr>
</thead>
</table>
| **Practical 1**       | **Microscopy** General     | **Description** You will be assigned to your group and discuss the responsibilities of each group member. The following role allocations may help with this process:  
Manager/Leader  
• getting the group organised  
• keeping the group on task  
• organising tasks into sub-tasks  
• making sure everyone has a chance to contribute  
Sceptic/Thinker  
• ensure the group avoids premature agreement  
• ask questions that will lead to understanding  
• push the group to explore all possibilities  
Checker/Recorder  
• check for consensus among group members  
• record the group’s solutions  
Conciliator/Explainer  
• resolve conflicts  
• minimise interpersonal stress  
• re-emphasise the main points  
• ensure each member understands what they have to do |
|                      | **discussion**             |                                                                                                                                              |
| **In your own time**  | **System & model**         | **Discussion** Discuss what biological system you would like to examine and possibly ideas for a model. All discussions within the group and with your tutor are to be recorded using the template available on Moodle. |
| **Practical 2**       | **Identifying cell types** | **Tutor review** During your lab discussion this week, you will discuss your biological system with your tutor for review. Your tutor will give you feedback on the appropriateness and feasibility of system, along with any suggestions for improvement. |
| **In your own time**  | **Research & design of**   | **system & model** During this time you will research and document all aspects of your biological system and commence/consider the design of your proposed model. |
| **Practical 8**       | **Genetics inheritance**   | **Tutor review** During your lab discussion this week, you will discuss your model with your tutor for review. Your tutor will give you feedback along with any suggestions for improvement. |
| **Practical 9**       | **Genetics screening by**  | **PCR** Your model should be near completed. During lab discussion time, you should show evidence of your design and discuss with your tutor. |
| **Practical 10**      | **Biological modelling**   | **You will present your model to the class in the Week 13 lab. You must also hand in the material documenting your discussion, with a group cover sheet. Be prepared to answer questions!!!** |

Further details on this assignment will be posted on Moodle and discussed in the practical class when your groups are formed. If you miss this assessment time due to illness or misadventure, please apply for special consideration online (see page 19 for instructions).
G. Mastering biology – 5%

In weeks 3, 7, 8, 10 and 11 you will access to an online quiz related to the concepts learned during the practical component of the course. Each quiz is worth 1% of your final assessment and should take you less than 30 minutes to complete (although it is not timed). The quiz will be accessible via Moodle on the week of the relevant practical and is due by the end of the following week (11:59pm on Friday of week 4, 8, 9, 11 and 12 respectively).

H. Final Theory Exam – 30%

The final examination is conducted externally during the UNSW June examination period. The exam can address ANY material covered in lectures and practical classes throughout the BABS1201 program. The format and weighting of questions in the final exam will be shown on the front cover of the exam paper, which will be posted on Moodle after submission to the exams branch part way through this session. PLEASE NOTE that the final exam is a COMPULSORY assessment and must be completed in order to satisfy the requirements for passing the course. If you miss this exam due to illness or misadventure, please apply for special consideration online (see page 19 for instructions).

Please note that there are NO PAST EXAM PAPERS provided in this course. The best ways to study for the above tests and exams are to revise your lecture notes and corresponding learning outcomes, using the mastering biology and other revision activities provided for you via moodle and access any resources that are referenced, including your recommended text for the course.
WEEK 1

COURSE INTRODUCTION
&
LABORATORY SAFETY

CONTENTS
1. Allocation of laboratory places
2. Introduction to course
3. Health & safety
4. Online Laboratory Safety Quiz
5. Meeting your classmates
6. Thinking like a scientist

1. ALLOCATION OF LABORATORY PLACES

Students should proceed to the appropriate laboratory (BioScience labs G20 and G21) where practical class groups will be organised.

2. INTRODUCTION TO COURSE

Your demonstrator will outline the scope of the course and explain the method of continual assessment to you and answer any questions you have about them.

3. HEALTH & SAFETY

This week you will meet your laboratory demonstrator, and be introduced to the basic principles of laboratory safety. This is a requirement of the Health and Safety legislation. At the end of the class, you will be required to sign a declaration that states that you have read and understood the rules. If you fail to do this, you will not be permitted to participate in further practical classes.

General conduct:

A laboratory is intended for serious work and rowdy behaviour is forbidden.

Students must read the instructions to their experiments carefully before starting work, and should be aware of all possible hazards.

No undergraduate students are to work in the laboratories outside class hours without permission and some degree of supervision.

All accidents and injuries must be reported to the lecturer or demonstrator in charge of the practical class, so that treatment may be provided if necessary and the incident reported.
Evacuation:

If there is a fire or other major calamity an alarm will sound. Messages may be broadcast from the university’s Emergency Response group. Unless there is an immediate danger nearby, when you first hear the initial ‘Prepare to Evacuate’ alarm, stop what you are doing and wait for further instructions.

Follow the instructions from your lecturer or demonstrator. Close all the doors and windows if possible. Quickly check to see that everyone is out of the room. Move steadily to the exit. If for some reason, you are not in the groundfloor labs, move quickly to the nearest stair well and out of the building. Do not use the lifts. **Assemble in the Michael Birt Gardens in front of the Chancellery Building (near Gate 9 on High Street).** Supervisors should bring the class roll and check that every one has left the building.

Risk assessment:

Working in a laboratory is inevitably associated with certain risks. Good laboratory practice means working in such a way as to eliminate, or at least minimise, these hazards. In order to perform your work safely and to comply with government legislation’s, a risk assessment has been conducted on all of work that will be performed in this subject in the laboratory and the following potential risks have been identified:

**Biological hazards:** All microorganisms are potentially harmful if ingested or exposed to body surfaces. Some organisms used in this class may be opportunistic human pathogens, however none are considered to pose a significant risk if handled appropriately (see procedures below).

**Chemical hazards:** Most of the chemicals used in this subject (eg. in solid and liquid media and most buffers) are not hazardous at the concentrations that are being used, however all chemicals should be considered potentially harmful. Some practicals employ hazardous chemicals. In these cases the hazard is described in the class directions for that specific exercise. The concentration of antibiotics in media are generally not harmful, however contact with skin should be avoided.

Note: Material Safety Data Sheets (MSDS) are available for all of the hazardous chemicals from your tutor. You should be familiar with the relevant MSDSs prior to commencing your practical work.

**Physical hazards:** Bunsen burners and heat from other sources such as water baths, breakable glassware, sharp objects such as plastic tips and needles.

**Hazards involving work environments:** The combination of large numbers of students performing laboratory work (eg. with Bunsen burners alight), and the necessity to wear protective clothing (see below), especially in summer weather, may cause discomfort to some students. In addition, the nature of laboratory design (benches and stools) may cause discomfort to some students.
Procedures for reducing risks:

In addition to the general risks that have been identified with laboratory work for this subject (see above), any additional risks associated with specific practicals are written in this manual at the beginning of each practical description. At the commencement of each new practical your tutor will review the risks with you. At the commencement of each class the procedures may also be reviewed. You may be examined on your understanding of such risks and their management. It is imperative that you be present at the beginning of each class to ensure that you are available to review safety procedures. If you are not present you may be excluded from the class. Below are some simple rules that you must follow which will ensure good laboratory practice and minimise the consequences of risks:

Wear adequate protective clothing. This will protect you from contamination by cultures and chemicals as well as protecting the cultures and chemicals from contamination by you. A laboratory coat must **ALWAYS** be worn while in the lab, and removed on leaving the lab. Where necessary (as advised by your tutor), protective gloves should also be worn. These will be provided and **MUST** be disposed of in the designated ‘Scientific Waste’ bins and **NOT** the general waste bins. **DO NOT wear lab coats or gloves outside the laboratory!** Adequate protective clothing also includes footwear. Fully enclosed shoes must be worn at **ALL** times while thongs and other open, loose footwear are not permitted. And if you have long hair, it must be tied back or up whilst you are in the laboratory. Safety glasses will be provided if required for certain procedures.

You must not eat, drink, smoke, apply make-up etc. in the lab. Neither should you bring food, drink etc. into the lab. **Never leave food or drink (water bottles included) on laboratory benches!** Habits such as chewing the ends of pens and pencils, nail biting etc. are often difficult to avoid, but you should make a conscious effort not to do them. Do not sit on laboratory benches. All bags and/or extraneous clothing items must be stored **UNDER** benches and **NOT** on benches or on the floor between the benches where they could act as a tripping hazard.

Do not invite anyone into the lab. They may not be aware of the hazards and may themselves create additional hazards.

Keep everything covered. Do not leave the plugs off flasks or caps off tubes and bottles. As well as minimising spillages, this will prevent contamination of cultures and solutions.

If there is an accident with a microbial culture, or hazardous chemical, ask a fellow student to call someone in authority immediately. Do not move and risk the spread of contamination. If there is a fire, remove yourself from immediate danger and call someone in authority immediately. If there is a small spill of a non-toxic or harmless chemical or solution such as water, you should clean it up yourself or check with your demonstrator first for the best way to proceed.

Before leaving the laboratory, tidy your bench, clean your bench area and **ALWAYS wash your hands.**

If you feel discomfort from your work (eg. heat exhaustion or back pain), consult your demonstrator or lecturer in charge.
If you get any biological or chemical substance (eg. sodium hydroxide) in your eye, ensure that you immediately go to a tap and wash your eye. While washing your eye, alert someone to your situation so that they can assist you and gain the attention of someone in authority. Continue to wash your eye until someone in authority indicates for you to do otherwise. It is imperative that you take this seriously as you may risk permanent eye damage if it is a harmful chemical. Note: you should always wear safety glasses when handling hazardous substances. These will be provided if required.

Acid splashes on the skin should be immediately washed thoroughly, and in the case of a major spill, you should douse yourself immediately using the safety showers. You should ensure that your demonstrator is aware of what has happened, and they may refer you to a school safety officer or medical officer.

**Using your knowledge of safe work practices in the laboratory, identify the problems in this picture:**

![Figure from: Baker, M. (2016) How quality control could save your science. Nature 529, pp 456–458 doi:10.1038/529456a](image-url)
You must sign the declaration below and have it witnessed by a tutor or demonstrator before you will be permitted to take part in practical classes.

I, ..........................................................................................................................
name .............................................................................................................

.................................         ........
student ID

certify that I have read and understood the Safety in Laboratories information above, and agree to abide by these rules at all times when in University Laboratories.

Tutor: ...........................................................................................................
name .............................................................................................................
signature

4. ONLINE LABORATORY SAFETY QUIZ

In order to be permitted to take part in laboratory classes from Week 2 onwards, you must also complete an online Laboratory Safety Quiz that is accessed through the BABS1201 Moodle site. Prior to your Week 2 laboratory class, go to the BABS1201 Moodle site and enter the ‘Laboratory Safety Quiz’ module by clicking on the appropriate icon on the home page. Follow the instructions provided there and use the above information on occupational health and safety that you have discussed with your demonstrator today to complete the quiz. When you have finished the quiz and submitted all your answers, you will receive a mark out of 12. If ANY of your answers were incorrect, then you MUST attempt the quiz again and keep repeating this process until you have scored a mark of 12/12 (that is, 100%). Since it is crucial that you are aware of all important health and safety rules and regulations, you can attempt the quiz as many times as required for you to get ALL the answers correct. Your final quiz mark will be checked prior to your lab in Week 2. If you have not scored 100% in the quiz by 9am on the day of your Week 2 practical class you will NOT be permitted to attend that lab class or any subsequent lab class until you have satisfied this requirement.
5. MEETING YOUR CLASSMATES

BABS1201 is one of the first courses many students undertake at university. To help you meet those you will work with and to help you learn skills for managing your workload, staying motivated and achieving well in your assessments there are some self-management activities in the practical classes. The skills practised in these activities will also help you be organised and resilient at university, in the workplace and other parts of your life.

Define Your Goals (5 min)

In a group of 2 or 3:

a. Choose a ridiculous goal e.g. go to space; learn how to juggle chainsaws; start an empire (30 sec):

b. Make the goal as specific as possible, and devise a way to measure the outcome:

c. Divide your goal into sub-goals (e.g. things that you will aim to achieve, or individual tasks that each need to be completed to achieve your goal). Add methods and deadlines.

Sub-goal/tasks: Method: Deadline: Resources needed:
1. 
2. 
3. 
4. 

d. Think about what resources you will need (prepare), and work out ways to get those resources. (perhaps add above).

e. Anticipate potential barriers/setbacks, write them down, brainstorm solutions, choose one and write it down. But also have backup plans.

f. Anticipate reviewing your goals and sub-goals/tasks periodically, and recognize that you might need to change your sub-goals and methods, and even your goal—a feasible alternative?
Video (4 min)
BABS1201 is one of the first courses many students take at university and many students find being 100% responsible for their own studies challenging. This exercise helps you to set academic goals and keep on track to achieve them.

Research has shown that students that set academic goals perform better! Watch this short video on goal setting: https://vimeo.com/120112496 (link also on Moodle in the Self-management section).

Self-management: Individual activity (3 min)
a. Write down at least one academic goal for this session.

b. Focusing on your academic goal (eg obtaining a credit in this course, handing in completed assessments on time), write down WHY you want to achieve this goal.

c. Rewrite the academic goal to be as specific as possible and focus it on this course - AND devise a way to measure the outcome (eg you intend to obtain a credit for each assignment in this course).

d. Divide your goal into sub-goals (eg milestones to achieve, or individual tasks that need to be completed to achieve your goal). Later, add methods and deadlines. You may need to check the assignment information in the front of this manual.

Sub-goal: Method: Deadline: Resources needed:
1. 
2. 
3. 
4. 

e. Think about what resources you will need (prepare), and work out ways to get those resources.

f. Anticipate potential barriers/setbacks, write them down, brainstorm solutions, choose one and write it down. But also have backup plans.

g. Anticipate when you will review your goals/sub-goals periodically, and recognise that you might need to change your sub-goals/tasks and methods, and even your goal— what might be a feasible alternative? e.g. (obtaining a pass in the course).

Do it! Finish this activity and you demonstrator will come and look through it for you to see how they can assist in keeping you on track. Further resources are available on the course Moodle site.
6. THINKING LIKE A SCIENTIST

Introduction:

Today you will start your training in how to think scientifically, and to evaluate whether information you come across is good value and reliable, or is merely someone’s opinion. This is a skill which will be important when you prepare assignments in this course. The exercises today are meant to make you think about how you evaluate information every day of your life, and how skills in the evaluation of information can be applied to the study of science and of scientific discovery.

Activity 1:

Divide into groups of 4-5. Your tutor will allocate one of the items in the list below to each group. If you were making a decision relating to the item you are given, how would you make an informed decision? You look at and evaluate a number of sources of information in order.

- Purchasing a mobile phone
- Whether to be vaccinated before going overseas
- Changing your diet to eat healthier
- Whether to take dietary supplements when starting an exercise regime

In your groups, discuss purchasing one of the above items, and analyse the processes you go through when doing so. Answer the following questions:

What sources of information do you use to help make your decisions?

How do you evaluate whether these sources are reliable or not?

If you find two sources of information that are conflicting, how do you decide which one to believe?
Activity 2:

As a group, work out the dimensions of the UNSW sign on top of the library building.

Please note: Do not take any risks such as climbing the building!

8. Introduction to Mastering Biology

The purpose of the following exercises is to familiarize you with the system you will be using for the rest of your course. These exercises are not intended to teach or test your knowledge of any specific subject material. Therefore, you will not be penalized for using hints or submitting incorrect answers.

Welcome to Mastering! Mastering presents homework items assigned by your instructor and works with you to answer them. Items typically have an introduction, possibly figures or animations, and one or more parts for you to answer.

1. Navigate to the Week 1 Practical via Moodle and complete the activity named “Introduction to Mastering Biology”.
2. If you have any trouble running the exercises you may need to adjust your browser settings, see here:

Your demonstrator must check that you can access Mastering Biology before you leave. If you do not have a device, you can borrow one in the lab. We need to ensure you can access the resources before they are used in class.
SECTION 1:

PRACTICALS 1- 3
EXPLORING CELL STRUCTURE

This sequence of three practical classes explores aspects of cell structure and concepts that will be discussed in lectures. You will learn some of the techniques we use to explore cells.

The goals for this sequence are:

- To become proficient at identifying and reporting on microscopic structures observed through a light microscope.
- To be able to identify an unknown cell type and report on your findings to your colleagues, including:
  - What type of cell (bacteria, eukaryote, plant or animal) you have.
  - What procedures you used to identify your cells.
  - What characteristics of those cells enabled you to identify them:
    - Organelles?
    - Size?
    - Other features or structures?
- To be able to record observations and results from your investigations in the manner of a scientist, including appropriate referencing of the scientific literature.

Graduate attributes:

- Research, inquiry and analytical thinking abilities
- Communication
- Information literacy
BABS Teaching Laboratory
Practical 1: Cell Structure 1
BABS_RA_BABS1201_1

<table>
<thead>
<tr>
<th>Hazards</th>
<th>Risks</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological agent (Microorganisms, Risk Group I)</td>
<td>Infection</td>
<td>Follow demonstrator’s instructions when handling biological agents.</td>
</tr>
<tr>
<td>Paramecium, Amoeba, Amoeba</td>
<td>Spills</td>
<td>PPE (lab coats, closed in shoes &amp; gloves as required).</td>
</tr>
<tr>
<td>Biological agent (Environmental samples – pond water)</td>
<td>Cuts from broken glass</td>
<td>Adhere to aseptic techniques.</td>
</tr>
<tr>
<td>Glass slides/cover slips</td>
<td></td>
<td>Notify demonstrator immediately of any spills or other incidents.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carry slides/cover slips in suitable containers and dispose of any</td>
</tr>
<tr>
<td></td>
<td></td>
<td>broken/used items immediately in sharps containers provided.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proper handwashing with antibacterial handwash before leaving the laboratory.</td>
</tr>
</tbody>
</table>

| Electrical Equipment (microscope) | Electric shock/ electrocution | Avoid water/spillages when working with electrical items. All equipment regularly tagged and tested by UNSW staff. Maintain good posture at all times whilst working at the microscopes and computers. Do not work at the microscope/computer for more than two hours without taking a break. |

<table>
<thead>
<tr>
<th>Ergonomics</th>
<th>Exposure to biological agents (see above)</th>
<th>Postural damage from extended periods of time working at microscope and computer</th>
</tr>
</thead>
</table>

In the event of an alarm sounding, stop the experiment, turn off electrical equipment and wait for confirmation from demonstrators to evacuate. Then pack up your bags and wash your hands. Follow the instructions of the demonstrators regarding exits and assembly points. The BioScience assembly point is in the lawn in front of the Chancellery. In the event of an injury inform the demonstrator. First aiders and contact details are on display by the lifts.

All used microfuge tubes should be placed in the biohazard bins. Used test tubes should be placed in the appropriate container on the front bench of the lab. All solutions should be placed in the appropriate liquid waste containers. Used slides and cover slips should be placed in approved biohazard sharps containers.

I have read and understand the safety requirements for this practical class and I will observe these requirements.

Signature:……………………………………………………………………. Date:…………………………
Student number:………………………….
1. DISCUSSION OF THE SCIENTIFIC LITERATURE ESSAY

Your first BABS1201 assignment is a reflective essay task that compares a scientific review, on a biological topic of your choice, with a primary research article.

Refer to pages 25-26 of this course manual for all detailed instructions on how to complete and submit this assignment.

At the beginning of today’s laboratory session, your demonstrator will summarise the key features and requirements of the essay assignment. So make sure that you familiarise yourself with the instructions so that you can ask questions and clarify any matters that you are unsure of today. The post-lab discussion at the end of today’s laboratory class will also give you a chance to further develop your understanding of primary and secondary scientific literature and the differences between them.
2. **PRINCIPLES OF LIGHT MICROSCOPY**

Light microscopes are a powerful tool for identifying and examining single cells or tissues. While there are many other techniques that can be used in conjunction with light microscopy, such as electron microscopy, DNA fingerprinting and biochemical techniques, light microscopes are still a crucial element of our scientific armoury, and are widely used.

For example:

- Hospital laboratories will look down a microscope to help identify a bacterial species, such as *meningococcus*, that is causing illness.
- Pathology laboratories will look at blood cells down a microscope to identify leukaemia’s, or tissue samples to identify other cancers.
- Ecologists may look down a microscope to identify the microscopic organisms present in the environment that can indicate the presence of pollution.
- Botanists use microscopes to identify seeds that are fertile.
- Biotechnologists may look down a microscope to identify cells that have successfully been engineered to express a desired protein.

The characteristics of individual organisms that can help identify them include size, shape, and internal structures. You can also use chemical stains to colour the organisms which can provide even more information. So knowing how to get the most out of your light microscope is a skill that you could need at many stages of your future career.

**The compound light microscope:**

The compound light microscope is a precision optical instrument designed for producing magnified images of objects using two or more glass lenses. The term “light” refers to the fact that light transmits the image to your eye, in contrast to ‘electron microscopes’ in which beams of electrons are used to create magnified images. “Compound” deals with the microscope having more than one lens. “Microscope” is a word created from "micro" meaning small and "scope" meaning view.

The key factor in optimising the compound light microscopes performance is not magnification, but resolution. Resolution is the ability to separate two closely spaced items. A lens magnifies by bending light. Optical microscopes are restricted in their ability to resolve features by a phenomenon called diffraction which, based on the nature of the optical system and the wavelengths of light used, sets a definite limit to the optical resolution. Due to the diffraction of light, even the best optical microscope is limited to a resolution of 0.2 micrometers. In other words, the smallest detail that can be seen under the highest magnification of the light microscope is 0.2 micrometers (µm).

When using the 100X lens the light is bent at such an angle as it passes from glass into air that it is impossible to properly or clearly observe the specimen. To prevent the light being bent away on an angled path from the objective lens, immersion oil is used. Immersion oil has the same refractive index as the glass, so light travelling up through the slide, the oil and then the objective lens, is not refracted again until it passes from the convex upper surface of the lens into the air above.
That bending, however, is what the lens is designed to do, sending the rays which left the specimen at angles up the tube at new angles, to be resolved and magnified by the ocular lens.

The oil immersion lens (100X), when used with a drop of oil, prevents this refraction or deflection of angled light from its straight path as would occur if the light were to pass at an angle from glass into air.

To use the oil immersion lens (100X), a drop of immersion oil is placed on the specimen and the oil immersion objective (100X) is then lowered into the oil.

Please note that immersion oil must not be used with any other lens (4X, 10X, 40X), as these lenses are not designed to come into contact with immersion oil, and the use of oil will result in damage to the lens.

**Parts of the light microscope:**

There are many makes and models of light microscope. However, all light microscopes are fundamentally the same, have similar controls and functions. The microscope illustrated below is typical of the light microscope used in UNSW teaching.
3. Setting up the light microscope

It is easy to view specimens with a microscope, but it is more difficult to obtain the best view possible. The following section takes you through a step-by-step process that will optimise the performance of the microscope for your eyes.

When you look through the eyepiece, try to keep both eyes open. If you have trouble, cover one eye with your hand. But commit yourself to keep trying. You must eventually be able to keep both eyes open.

1. Switching on microscope

Turn the power switch on (1).

Move the voltage control slide (2) to set the light intensity. You should not need to set the intensity to the maximum power.

2. Specimen placement

Open the spring-loaded finger of the specimen holder (1) and insert the slide that is provided for each student.

3. Focus

Swing in the 4x objective (1).

Using the coarse adjustment (2), raise the stage as high as possible. Bring the specimen into focus by lowering the stage, using first the coarse and then the fine adjustment knobs.

Swing in the 10x objective and refocus using the coarse and/or fine adjustment knobs.
4. Interpupillary distance

Looking through both eyepieces, move the knurled dovetail slides until a suitable binocular image is obtained.

5. Diopter adjustment

To achieve maximum binocular clarity, an adjustment can be made to compensate for differences in the vision of your left and right eyes. Look at the image through the right eyepiece with your right eye, and focus on the specimen with the fine focus adjustment.

Looking at the image through the left eyepiece with your left eye, rotate the diopter adjustment ring (1) to focus on the specimen without using the focus adjustment knobs.

6. Adjusting the height of the condenser

Raise the condenser using the condenser height adjustment knob (2) until it is as close to the slide as possible. Then lower the condenser until the ‘pearly’ image of the ground glass of the lamp unit is sharply visible in the field of view. Now readjust the focus to put the condenser slightly out of focus – the ground glass surface should just disappear. The condenser can only be focused correctly when the object on the slide is itself in focus.

7. Objective change

Swing in the 40x objective to examine your slide at relatively high magnification. Ensure that the nosepiece is clicked into position.
Evaluating your microscope set-up:

Once your microscope is set up to your satisfaction, leave it on 40X objective, and have your tutor/demonstrator check it.

Microscope adequately adjusted

Oil immersion

When you need higher magnification....

The 40X objective gives you about as good a magnification as you can get with a lens-in-air. Higher magnification just gives you larger, blurred images. However, resolution at higher magnification (100X objective) can be achieved if light from the specimen passes to the lens through clear oil rather than air, because light scattering at an oil/glass interface is less than at an air/glass interface. The oil immersion lens (100X), when used with a drop of oil, prevents refraction or deflection of angled light from its straight path that would occur if the light were to pass at an angle from glass into air. The degree to which the light is refracted or bent by a substance is formulated as its refractive index. As you might expect, the numerical aperture of a lens, the light-function constant you used to calculate the resolution, is determined in part by the refractive index of the glass.

To prevent the light from being bent away on an angled path from the objective lens, and to allow the maximum amount of light from the specimen to be gathered by the objective, a drop of immersion oil may be placed on the specimen and the 100x oil immersion objective can then be lowered into the oil.

Immersion oil has the same refractive index as the glass, so light traveling up through the slide, the oil and the objective lens is not refracted again until it passes from the convex upper surface of the lens into the air above. That bending, however, is what the lens is designed to do, sending the rays which left the specimen at angles up the tube at new angles, to be resolved and magnified by the ocular lens.
As light strikes the specimen the qualities of the light are changed in several ways that give the visual image we perceive. It may be scattered or reflected away from a path leading to the objective, darkening the image; it may be completely occluded by solid structures that appear black to the observer; specific wavelengths of the light may be partially absorbed by certain substances (including stains), giving a characteristic colour to structures containing them.

**Microscopy trouble shooting**

<table>
<thead>
<tr>
<th>Apparent fault</th>
<th>Possible cause</th>
<th>Correction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field dark</td>
<td>Power (lamp) not on or turned down too low</td>
<td>Turn power on &amp; check voltage</td>
</tr>
<tr>
<td></td>
<td>Condenser diaphragm closed</td>
<td>Open diaphragm</td>
</tr>
<tr>
<td></td>
<td>Lamp filament burnt out</td>
<td>Replace lamp</td>
</tr>
<tr>
<td>Colour of objects indistinct</td>
<td>Condenser diaphragm closed too far</td>
<td>Open diaphragm</td>
</tr>
<tr>
<td>Poor resolution</td>
<td>Condenser either too far open or too far closed</td>
<td>Adjust condenser diaphragm</td>
</tr>
<tr>
<td>Unable to focus on object</td>
<td>Cover-slip too thick</td>
<td>Replace</td>
</tr>
<tr>
<td></td>
<td>Slide up-side down</td>
<td>Invert slide</td>
</tr>
<tr>
<td></td>
<td>Focusing attempts too rapid</td>
<td>Use fine focus and adjust more slowly</td>
</tr>
<tr>
<td></td>
<td>Objective has insufficient resolving power</td>
<td>Use higher power</td>
</tr>
<tr>
<td></td>
<td>Objective covered with dried immersion oil from previous use</td>
<td>Clean with lens tissue and solvent</td>
</tr>
<tr>
<td>Specks in field of view</td>
<td>Dirt on eye lens of ocular</td>
<td>Clean with lens tissue</td>
</tr>
<tr>
<td></td>
<td>Dirt on condenser lens</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dirt on filter</td>
<td></td>
</tr>
<tr>
<td>Moving shadows in field</td>
<td>Air and/or water bubbles in immersion oil</td>
<td>Remove oil with lens tissue. Re-apply</td>
</tr>
<tr>
<td>Light suddenly reduced</td>
<td>No oil contact between oil immersion objective and slide</td>
<td>Adjust with course /fine focus control</td>
</tr>
</tbody>
</table>

Calculating the total magnification of an image that you are viewing through the microscope is really quite simple. To get the total magnification, take the power of the objective (4X, 10X, 40X) and multiply by the power of the eyepiece to give total magnification.

If you are looking at something through the 40X objective, what is the actual magnification of the object you see?
Measurements and scales when using microscopes:

Often it is sufficient just to know an approximate measurement of the size of the object you are viewing, i.e. whether it is about 10 µm or 10 mm in diameter. And it is important to realize that the apparent size of the specimen will naturally depend on which objective lens you are using to view it.

A good way to obtain an estimate of the size of the object is by comparing it to the diameter of the field of view.

The diameter of the field of view is dependent on the magnification of two lenses - the eyepiece lens and the objective lens. Since the eyepiece lens remains unchanged, we can take the diameter of the field of view of the eyepiece and modify it for the various objective lenses. The field number of the eyepieces you will be using is 18 mm.

To calculate the true diameter of the field of view with different objectives, we use the formula:

\[
\text{diameter} = \frac{\text{field number}}{\text{objective magnification}}
\]

So, using the 10X objective:

\[
\text{diameter} = \frac{18}{10} = 1.8\text{mm}
\]

Therefore an object on the slide which occupies half the field of view will measure approximately 0.9 mm or 1 mm across.

Accurate measurements using the eyepiece scale

In order to provide an accurate scale for a drawing it may be necessary to have accurate measurements. A ‘ruler’ or micrometer is built into the eyepiece of your microscope. A microdot slide of the number ‘5’ has been provided as a trial slide.

**PLEASE NOTE:** make sure that your slide is positioned with the correct side facing upwards. If you cannot focus on the number ‘5’ at higher objectives, you may have the slide upside down! With this slide on the stage and in focus, observe the eyepiece micrometer. Notice that the scale has 100 divisions.

Starting with the lowest power, move through the different non-oil objectives and note that the size of the ruler does not change. However the apparent size of the ‘5’ changes with each new objective lens used. Therefore the divisions of the ruler include a different amount of the ‘5’ with each different objective.

The high quality of your microscope lenses is such that calibration made at the factory are good for all microscopes of the same model.
The values are:

<table>
<thead>
<tr>
<th>Objective</th>
<th>Eyepiece Scale Division</th>
</tr>
</thead>
<tbody>
<tr>
<td>4X objective</td>
<td>25 µm</td>
</tr>
<tr>
<td>10X objective</td>
<td>10 µm</td>
</tr>
<tr>
<td>40X objective</td>
<td>2.5 µm</td>
</tr>
<tr>
<td>100X objective (oil)</td>
<td>1 µm</td>
</tr>
</tbody>
</table>

You can get an accurate measurement of any object on a slide at any magnification. Test this by measuring the '5' on the microdot slide.

Using the scale, how high and how wide is the '5'?

Now you know the size of the object seen through your microscope. However, someone looking at a drawing you made of it will have absolutely no idea of its actual size unless you also include some indication of size. This is done by placing a scale on the drawing.

4. OBSERVING MICROSCOPIC LIFE

Experimental procedure:

1. Obtain a small sample of the pond water provided.

2. Place a drop on a clean microscope slide, and gently lower a coverslip onto the slide as shown in Figure 1 below. This provides some protection for your specimen. It prevents the specimen drying out, and it allows you to place oil on top of the coverslip when you wish to use the oil immersion lens. Be careful not to squash your specimen.

   Figure 1: Placing a coverslip on a slide.

3. Look at your slide under low power.

   How many different types of organisms can you see?

   How many are unicellular and how many multicellular?
Can you tell whether they are plants or animals? What characteristics might help you decide?

Some common protists:

Protists are an informal term that describes a heterogeneous group of living things, comprising those eukaryotes, which are neither animals, plants, nor fungi. Most protists are unicellular eukaryotic cells, resembling animals and plants, and differing from bacteria, for they have at least one well-defined nucleus. If your pond water slide did not contain any protists, please take time to examine some of the fascinating organisms that have been grown in the laboratory for you. Hopefully we will have living specimens of *Amoeba, Euglena* and *Paramecium* for you to examine. These are not easy organisms to maintain in a lab culture, so we may have to substitute other species. Look at the demonstrations of pond water organisms that are set out around the lab.

*Paramecium* are 'ciliates' (protists that use cilia for locomotion). Sometimes we have a species which is decidedly green. The green colour comes from the chloroplasts of symbiotic green algae that live within the *Paramecium*.

*Amoeba* is a genus of rhizopods (protists that use pseudopodia for locomotion). These are fascinating organisms to watch.

*Euglena* is a genus of green flagellates (protists that use a flagellum for locomotion). They are green because they contain chlorophyll and therefore can live by photosynthesis. However if placed in the dark they can also feed by ingesting food particles.
5. REPORTING YOUR FINDINGS

One good way to report to others what you see down a microscope, is to draw what you see. You do not need to be artistic, just accurate and clear.

Make sure you recognise and label important characteristics of the cells you see. This record might help you identify unknown cell types in later weeks (e.g. organelles, membranes, size, etc.)

Guidelines for drawing:

Drawing remains an important method of recording biological observations. It is also a useful thing to do since it encourages the observer to look more carefully at the specimen. For this reason, learning to produce good accurate drawings of your material is an important part of practical work.

Outline drawings:

These drawings show relationships between parts of the subject, but provide little detail. When using a microscope, line drawings are usually made to record what is seen with the low power objective lens.

See Figure 2 below.

Figure 2: Paramecium 100x magnification
High power drawings:

These drawings are made with the use of high power objective lenses and show individual cells. High power drawings may also show intracellular detail. To see any structure within a cell requires a high power objective lens, usually with oil immersion.

See Figure 3 below.

![Figure 3: Paramecium 1000x magnification (oil immersion)](image)

These two types of drawing can be combined in order to show high power detail in only a section of a specimen being illustrated. It would be extremely time consuming to show details of individual cells throughout a drawing of a large section, so it may be better to do a detailed drawing of one part only. In this case, the section which is to be drawn in detail must be clearly defined on the outline drawing.

When making drawings of microscopic specimens, many people prefer to use one eye to look down the microscope with the other eye focused on the drawing paper placed at the side of the microscope. With a bit of practice it is possible to draw and look down the microscope simultaneously. Always draw what you can see, not what you think you should see. You will find that habitual accurate drawing will increase your powers of observation. *All drawings should be completed in class. Never make a rough sketch and smarten it up later.* This always leads to inaccuracies.

Make your drawings large and clear using the space provided. Drawings should be made with a sharp HB pencil and the lines should be continuous. Never draw with pen or coloured pencils.
Where specimens have repetition of detail it is best to make an outline sketch of the whole specimen or field of view and then illustrate a clearly defined part of this sketch with a separate detailed drawing as illustrated in figures 2 and 3.

- Label the drawings and diagrams fully in pencil.
- Keep your labels horizontal and to the side of the drawing, and rule lines to the appropriate parts.
- Do not use arrowheads.
- Provide a title for each drawing.
- If notes are necessary as part of your observations, place them at the bottom of the drawing or near the appropriate label. This allows a combined record of structural and functional observations.
- There should be a scale with each drawing to indicate size.

**Your observations:**

Make line drawings as figures 4 and 5, and work out the actual size of any two of the organisms you see down your microscope.

Figure 4:
Re-adjusting your microscope:

To help you remember how to adjust your microscope, a second short video will be played.

On completion of microscopic examination:

When finished with your microscope, before returning it to the cupboard, always:

- If appropriate, clean oil from the oil immersion lens and from other lenses too if they have been contaminated with the oil.
- Return the light intensity to the lowest setting and then switch off.
- Rotate the nosepiece back to the 4X position.
- Remove any slide from the stage.
- Dry any liquid from the stage.
- Secure the power cord.

N.B. You must clean your lenses using the lens tissue provided: Never use ordinary Kleenex, as this can scratch lenses.

PLEASE NOTE that you will be assessed on your ability to correctly handle, adjust and pack away microscopes during your final practical exam in the course, so make sure that you follow the above directions very carefully at all times.
6. POST-LAB DISCUSSION: SCIENTIFIC LITERATURE

Activity 1:

Working in groups of 3-4 students. You will be provided with a package of three articles. You should spend about 5-7 minutes preparing to describe to the larger group, the subjects, sources and reliability of the articles. It will be impossible to closely read the entire set of articles in the time given. Rather, you must scan the documents to extract the critical information.

Your group should be prepared to describe their articles in just 2 minutes.

Activity 2:

Each group of 3-4 students will be given a journal. Flick through the journal, to get a sense of the purpose of the journal, and the structure and style of articles in the journal.

After some time, you will be asked to report your findings back to the larger group.

7. SELF-MANAGEMENT: ASSESSMENT PREPARATION

Which assessments are due in the next two weeks (over all your courses)?

Which assessments are due in the next month?

1. If you have not done this elsewhere, use the checklist below to write down a to do list of all the tasks you have to do this week towards your next assessments.

   Task checklist for this week:
   ☐
   ☐
   ☐
   ☐
   ☐
   ☐
   ☐
   ☐
   ☐
   ☐
   ☐
   ☐

2. How will you feel if you accomplish all these? What reward will you give yourself?

3. What is your overarching academic goal for this semester?

4. Are your checklist tasks suffice to help you move towards this goal?

5. Choose one of your upcoming assessments and discuss with your lab partner two sub-goals you can set to help prepare for this. Make them specific and measurable.
6. Come back to this worksheet **next week** to see how you fared—fill in the right side of the table.

<table>
<thead>
<tr>
<th>This week’s sub-goal / task two:</th>
<th>Did you complete it? Why / why not?</th>
</tr>
</thead>
<tbody>
<tr>
<td>How can you break this task down?</td>
<td></td>
</tr>
<tr>
<td>How are you measuring your progress?</td>
<td></td>
</tr>
<tr>
<td>What resources do you need?</td>
<td></td>
</tr>
<tr>
<td>What potential barriers may arise?</td>
<td></td>
</tr>
<tr>
<td>How will you deal with these barriers?</td>
<td></td>
</tr>
</tbody>
</table>

Next week: check in with your lab partner and see how they went on preparing for their assessment.
<table>
<thead>
<tr>
<th>Biological agent</th>
<th>Infection</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Microorganisms, *Bacillus</td>
<td></td>
<td>Follow demonstrators instructions when handling biological agents.</td>
</tr>
<tr>
<td><em>megaterium</em>)</td>
<td></td>
<td>PPCE (lab coats, closed in shoes &amp; gloves as required).</td>
</tr>
<tr>
<td>Biological agent (Environmental</td>
<td></td>
<td>Adhere to aseptic techniques.</td>
</tr>
<tr>
<td>samples – <em>Spirogyra</em>)</td>
<td></td>
<td>Notify demonstrator immediately of any spills or other incidents.</td>
</tr>
<tr>
<td>Razor blades, glass slides/cover-</td>
<td></td>
<td>Carry slides/coverslips in suitable containers and dispose of any</td>
</tr>
<tr>
<td>slips</td>
<td></td>
<td>broken/used items immediately in sharps containers provided.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proper handwashing with antibacterial handwash before leaving the</td>
</tr>
<tr>
<td></td>
<td></td>
<td>laboratory.</td>
</tr>
<tr>
<td>Hazardous chemicals (nitroblue</td>
<td></td>
<td><strong>Personal Protection</strong>: Wear appropriate safety goggles, gloves and</td>
</tr>
<tr>
<td>tetrazolium salt)</td>
<td></td>
<td>protective clothing.</td>
</tr>
<tr>
<td></td>
<td>Eyes: Causes irritation.</td>
<td><strong>Eyes</strong>: Immediately flush with plenty of water for at least 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>minutes.</td>
</tr>
<tr>
<td></td>
<td>Skin: Causes irritation.</td>
<td><strong>Skin</strong>: Immediately flush area with soap and water.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Label <strong>Hazard Warning</strong>: Caution! May cause eye and skin irritation.</td>
</tr>
<tr>
<td></td>
<td>Ingestion: Harmful if swallowed</td>
<td>May cause respiratory and digestive tract irritation.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inhalation: Causes respiratory tract irritation.</td>
<td></td>
</tr>
</tbody>
</table>

**Closed in Footwear**

**Lab. Coat**

**Gloves**

**Safety glasses**

In the event of an alarm sounding, stop the experiment, turn off electrical equipment and wait for confirmation from demonstrators to evacuate. Then pack up your bags and wash your hands. Follow the instructions of the demonstrators regarding exits and assembly points. The BioScience assembly point is in the lawn in front of the Chancellery. In the event of an injury inform the demonstrator. First aiders and contact details are on display by the lifts.

All used microfuge tubes should be placed in the biohazard bins. Used test tubes should be placed in the appropriate container on the front bench of the lab. All solutions should be placed in the appropriate liquid waste containers. Used slides and cover slips should be placed in approved biohazard sharps containers.

I have read and understand the safety requirements for this practical class and I will observe these requirements.

Signature: ___________________________________________ Date: ____________________________

Student number: __________________________
OBJECTIVES

- To identify key differences between eukaryote and bacterial cells, including their size and structure.
- To identify intracellular structures of an example of a eukaryotic cell.

CONTENTS

1. Internal structure of an example of a eukaryotic cell, Spirogyra
2. Structure of an example bacterial cell
3. Comparison of size of eukaryotic and bacterial cells
4. Staining mitochondria in eukaryotic cells
5. Post-lab discussion – Team Enzyme Project

1. INTERNAL STRUCTURE OF A EUKARYOTIC CELL (SPIROGYRA)

Introduction:

In this exercise, you will examine a water sample containing Spirogyra. Note that we are not providing you with a pure culture. Samples were collected from local ponds and so will contain a range of protists, plants and animals.

All living cells can be divided into three main types: bacteria, archaea and eukaryotes. Bacteria and archaea are very similar in structure and were once collectively termed prokaryotes. Animals, plants and fungi are eukaryotes. There are many differences between the cell types, but the most obvious is that bacteria and archaea do not have a nucleus, whereas eukaryotes do. Also, as you are learning in lectures, eukaryotes have internal structures called organelles. Other important differences cannot be seen down a light microscope.

Spirogyra is a green algae (eukaryote), composed of cells arranged in long unbranched filaments. Each cell contains one or more long, green ribbon-like chloroplasts that wind around the periphery of the elongated cells.

As well as its distinctive ribbon-shaped chloroplast(s), Spirogyra has a cell wall, and a large central vacuole, and at its centre it has a nucleus. The degree of coiling of the chloroplast(s) seems to vary a great deal in different filaments, from being almost straight and lying along the long axis of the cell to being tightly coiled.

The nucleus and pyrenoids (organelles in or extending from the chloroplast and associated with reserve food accumulation) in the Spirogyra cell are normally colourless. They can be stained with iodine to make them more visible but this kills the cells.
Discovering the general structure of *Spyrogyra*:

1. Place a small drop of the solution containing *Spyrogyra* onto a clean microscope slide, and cover with a coverslip. If there is too much water, carefully blot off the excess with tissue paper, without squashing the material under the coverslip.

2. Place the slide on the microscope stage and examine it. Find a *Spyrogyra* filament with coiled chloroplasts using the 10X objective. You will not see much detail.

Note how the cells are joined to form the filament.

*Do the filaments branch?*

3. Focus on an individual cell with the 10X objective. Have a look at the general layout of the cell. See if you can identify the cytoplasm around the edge of the cell, the large central vacuole, the nucleus and the coiled ribbon-like chloroplast(s).

4. Make a line drawing of a chain of at least three *Spyrogyra* cells, and put a scale on it (figure 1). Provide a title for your figure. The aim of this drawing is to illustrate accurately the overall shape of the organism, the shape of each cell and how the cells are joined to one another. Do not show any structures within the cells.

N.B. Don’t let your specimen dry out completely. If it looks as though it might be drying, add another drop of pond water. You do not need to remove the cover-slip - just add the drop carefully at the side.

*Figure 1:*
Investigating chloroplasts in *Spirogyra*:

5. Now look at the chloroplasts in detail with the 40X objective. By shifting the fine focus up and down, check that they really are ribbon-like. These chloroplasts are quite different in shape to those of flowering plants which are usually disc shaped, and chloroplasts of other green algae have other shapes. Notice that at intervals the chloroplasts contain small shiny globules: these are called pyrenoids. They are ensheathed by starch and will stain with iodine.

Determine, by focusing, the number and position of the chloroplasts in the cell. Record your observations on the following page.

<table>
<thead>
<tr>
<th>Is there one chloroplast or several per cell?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Is/are the chloroplast(s) in the centre of the cells or at their edge?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Are there different numbers of chloroplasts in the different cells of a filament?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Do the chloroplast(s) continue from cell to cell?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

6. In a cell where the chloroplast(s) are tightly coiled, work out the direction of coiling (right-handed or left-handed helix) by varying your plane of focus. To do this it is necessary to determine when you are in focus in the upper plane and when you are in focus in the lower plane, and then focus through the top, middle and bottom of the cell.

Record your findings by making simple diagrams (Figure 2) indicating the appearance and position of a chloroplast ribbon at each of the three levels of focus in the cell to illustrate the direction of coiling.

Figure 2:
Investigating other internal structures of *Spirogyra*:

7. Prepare a fresh specimen by placing a drop of pond water on a fresh slide. Add a drop of iodine stain to it and wait for 4-5 mins then cover with a cover-slip. It is not necessary to wash out the stain.

8. Find a cell with a visible nucleus. The nucleus is irregular in shape and almost transparent and it is situated more or less in the centre of the cell. It usually contains a shiny spherical body called the nucleolus. Careful focusing will show the cytoplasmic strands that suspend the nucleus in the vacuole.

9. Make a drawing of the *Spirogyra* cell in the plane of focus of the nucleus using the 40X objective (Figure 3). You should be able to identify and label the cell wall, the position of the cell membrane, the cytoplasm, the vacuole, the nucleus, the nucleolus, the cytoplasmic strands that suspend the nucleus, the focused parts of the chloroplasts and their pyrenoids. Parts of adjacent cells should also be indicated, and you should include a scale. You should also provide a legend for your figure.

Figure 3:
2. STRUCTURE OF A BACTERIAL CELL

Bacterial cells have a much simpler structure than eukaryotic cells, and lack a nucleus or organelles. They are also usually smaller, although in this practical you will examine an unusually large bacterium, *Bacillus megaterium*. Since bacterial cells are almost colourless, you need to stain them in order to see them.

1. Shake the container of *Bacillus megaterium* to suspend the bacteria in the solution.

2. Place one small drop of the milky suspension on a clean microscope slide and label it with your name. Spread the drop by gently rocking from side to side.

3. Place the slide on the side bench under the lamps and let the drop dry completely (otherwise the bacteria will wash off the slide during subsequent procedures). While the slide is drying, proceed to section 3 “Comparison of size of eukaryotic and bacterial cells”.

4. When absolutely dry, add 1 drop of toluidine blue to the slide. After 5 minutes rinse off the stain and then dry it completely under the lamps. Once dry, your preparation is ready for examination by light microscopy.

5. Focus on the plane of the smear using the 10X objective. Then change to the 40X objective. When the bacteria are in focus, swing the 40X objective out of the way and place a drop of immersion oil on top of the bacterial smear. Now gently bring the 100X objective into place. Only a slight adjustment of the fine focus knob should be needed to view the *Bacillus* clearly.

6. Look at your bacterial cells and make a simple outline drawing (Figure 4).

Note: Bacterial cells are usually either rod-shaped or round, and may form clumps or chains when growing in suspension.
3. COMPARISON OF SIZE OF EUKARYOTIC AND BACTERIAL CELLS

In order to compare the sizes of your eukaryotic and bacterial cells, you need to measure them carefully. You can start by measuring *Spirogyra* while your bacterial smear is drying.

1. Measure the length and width of about 10 mature *Spirogyra* cells, and from these measurements, calculate the average cell length and width. (Record your measurements and calculations).

Assuming a *Spirogyra* cell is a regular cylinder and the ‘width’ measurement is a true diameter, calculate the average volume of the cell in the space below:

2. Using the 100X oil immersion objective, make similar measurements on the *Bacillus* cells. (A word of caution: be sure that you are only measuring the length of one bacterium, not a group or chain of bacteria). Using the same assumptions as for *Spirogyra*, calculate the average cell volume below:
4. STAINING MITOCHONDRIA IN EUKARYOTIC CELLS

Introduction:

Mitochondria are not easily seen in living cells, but it is possible to increase their visibility by staining them in a solution of pale yellow tetrazolium salt. This is a vital stain which enters the cells and is reduced in the mitochondria to form an intense coloured compound (purple or blue).

Safety note:

Tetrazolium salts are hazardous, so keep them off your skin. Disposable latex gloves will provided for your use. Make sure that you place used or unused gloves in the ‘Scientific Waste’ bins provided. NEVER leave ANY gloves on the bench or place them in non-‘Scientific Waste’ bins. Thank you.

Procedure:

Note: This procedure needs to be done carefully, and includes a 1 hour incubation. Make sure you set this up at the beginning of your class. While the cells are staining, you can proceed with other tasks.

1. Place about 10 drops of the tetrazolium solution into the container provided on your bench. Immediately cover the container with foil to protect it from light, as tetrazolium salts are sensitive to light and rapidly decay.

2. Cut several thin slices of surface tissue from the terminal centimetre of the broad bean root tip with a razor blade, and place them immediately into the solution in the container. Cover the container again and allow 1 hour for the reaction to take place.

3. After one hour, mount a piece of tissue, using water and cover-slip, and examine the thinnest part under the 10X and 40X and, if possible, the 100X oil immersion objectives. It may help if you close down the condenser diaphragm to get higher contrast. The mitochondria should appear as dark elongated or more or less round structures, about the size of bacteria, throughout the cytoplasm.
Make a simple drawing of a cell and the mitochondria (Figure 1).

Look at the electron micrographs of mitochondria available in the lab.

What structures can you see here that you cannot see under your microscope?

Why do you think you can’t see such structures in the cells you stained?
5. Mastering Biology Quiz

Don’t forget that your Mastering Biology Quiz 1 “Prokaryotic versus Eukaryotic Cell Structure” must be completed by the end of next Week 4. The quiz is worth 1% of your final assessment and should take you around 15 minutes to complete.

6. POST-LAB DISCUSSION: TEAM ENZYME PROJECT
Experimental Protocol, Results and Report

This task is a course assessment component and is worth 15% of your final mark in BABS1201. In order to complete this task, you will be assigned a lab partner so that together you form a smaller ‘team’ within your demonstrator group.

The project has three main stages or deadlines that you should become familiar with, as listed below:

2. Experimental Method, Results and Notes: SCHEDULED/DUE Practical 6, WEEK 9.

Refer to pages 27-34 of this course manual for full detailed instructions on how to complete and submit this assignment.

Towards the end of today’s laboratory session, your demonstrator will summarise the key features and requirements of the Team Enzyme Project assignment. You will also be placed in a team and provided with a specific enzyme to work on. So make sure that you familiarise yourself with all the instructions so that you can ask questions and clarify any matters that you are unsure of today.
### Student Risk Assessment

<table>
<thead>
<tr>
<th>Biological agent</th>
<th>Infection</th>
<th>Follow demonstrator’s instructions when handling biological agents.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Microorganisms, <em>Bacillus megaterium</em>)</td>
<td>Spills</td>
<td>PPCE (lab coats, closed in shoes &amp; gloves as required).</td>
</tr>
<tr>
<td>Biological agent (Environmental samples – <em>Spirogyra</em>)</td>
<td>Cuts from broken glass</td>
<td>Adhere to aseptic techniques. Notify demonstrator immediately of any spills or other incidents.</td>
</tr>
<tr>
<td>Glass slides/cover slips</td>
<td></td>
<td>Carry slides/cover slips in suitable containers and dispose of any broken/used items immediately in sharps containers provided.</td>
</tr>
<tr>
<td>Hazardous chemicals (nitroblue tetrazolium salt)</td>
<td>Eyes: Causes irritation.</td>
<td>Proper handwashing with antibacterial handwash before leaving the laboratory.</td>
</tr>
<tr>
<td></td>
<td>Skin: Causes irritation.</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Inhalation: Causes respiratory tract irritation.</td>
<td></td>
</tr>
</tbody>
</table>

**Personal Protection:** Wear appropriate safety goggles, gloves and protective clothing. **Eyes:** Immediately flush with plenty of water for at least 15 minutes. **Skin:** Immediately flush area with soap and water. **Label Hazard Warning:** Caution! May cause eye and skin irritation. May cause respiratory and digestive tract irritation.

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I have read and understand the safety requirements for this practical class and I will observe these requirements.

**Signature:** ................................................................. **Date:** .......................................................... **Student number:** ..........................................................
WEEK 4
PRACTICAL 3
CELL STRUCTURE III
DIFFERENCES BETWEEN PLANT CELLS & ANIMAL CELLS
DIFFERENCES BETWEEN BACTERIA & EUKARYOTES

OBJECTIVES
Living cells come in a range of shapes, sizes and degrees of complexity. In this practical you will perform a procedure commonly used to assist in the identification of cells - chemical staining. Different types of stains react with different kinds of molecules, and so cell structures may or may not stain with different coloured stains. We can use this to identify cell structures and compartments. There are two general ways of staining cells. They may be stained when they are alive (“vital stains”) or when they are dead. Cells are often chemically ‘fixed’ – a technique that prevents decay and preserves important features of the cells. You will use both types of stain in this class. You will also remind yourself of the main differences between bacterial cells and eukaryotic cells (plants, fungi and animals).

- To identify key differences between internal structures of prokaryotic and eukaryotic cells
- To become competent with basic staining procedures and light microscopy of different types of cells
- To identify and report on key internal structures of a range of cell types.

CONTENTS
1. Staining nuclei in animal and plant cells
2. Other structures of eukaryotic cells: cell walls, vacuoles & chloroplasts
3. Other internal structures of eukaryotic cells
4. Staining bacterial cells
5. Staining of fixed cells
6. Post-lab discussion – Scientific method
1. **STAINING NUCLEI IN ANIMAL AND PLANT CELLS**

In this procedure you will be using a stain, toluidine blue, that stains the nucleolus purple (indicating the presence of RNA) and the nucleus pale blue (DNA).

**Procedure:**

1. Lightly scrape the inside of your cheek with the wooden stick provided. This allows you to collect some of the so-called ‘epithelial cells’ that line many surfaces of the body.

2. Mount the scrapings directly on a microscope slide, and add a drop of toluidine blue solution. Leave 5 minutes for stain to work.

3. Cover with a coverslip and examine under 10X and 40X objectives.

   *Observe individual epithelial cells: what can you see?*

4. Compare your cheek cells with the prepared slide of a corn root tip. In this preparation, you will find many nuclei in various stages of division, but concentrate on cells with nuclei that are not dividing, i.e. nuclei that have a clearly defined circular outline and contain one or more nucleoli.

   *Can you see clear gaps between the cells in your corn root? What might this be?  Hint: what feature do plant cells have that animal cells don’t?*

   *What are the major differences between the plant cells and your cheek cells (animal cells)? What are the common features?*
5. Draw two cells from both the cheek cell and the corn root preparations to show their shape (Figure 2). Include their nucleus and any other internal structures that you can see, making particular note of differences. Label your drawings and include a scale.
2. **OTHER STRUCTURES OF EUKARYOTIC CELLS:  
CELL WALLS, VACUOLES AND CHLOROPLASTS**

**Procedure:**

You could not see details of the cell wall in your toluidine stained slides. Look at the demonstration slide stained specifically to show the carbohydrate rich cell walls.

1. Plant cells often contain a central vacuole. Look at the electron micrographs of vacuoles in plant cells. (What is a vacuole?)

   *Can you see central vacuoles in your corn root slides? Explain why this might be so.*

2. With the forceps provided, carefully mount a young leaf of the water plant *Egeria* in pond water, and examine with the 10X and 40X objectives.

   *Can you find chloroplasts?*

   *What internal structures can you see in the chloroplasts?*

   *How big are they?*
3. Record your findings as a drawing with a scale (Figure 3).

Figure 3:

4. Leave your Egeria cells for a few moments to recover from the shock of being removed from the plant, and look at it again.

What differences can you see?

5. Record your observations, and make a simple drawing of the main features that you see (Figure 4).

Figure 4:
3. OTHER INTERNAL STRUCTURES OF EUKARYOTIC CELLS

There are other internal cell structures that are not visible down a light microscope. Look at the electron micrographs that are available, and identify as many of the features as you can. Record the structures that you recognise, and identify their main function in Table 1.

Table 1:

<table>
<thead>
<tr>
<th>Structure</th>
<th>Function</th>
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4. STAINING BACTERIAL CELLS

Because bacterial cells are generally smaller than eukaryotes, and have a less well defined internal structure, it can be difficult to see them well under light microscopy, unless you use the high power (100X) oil immersion lens. Today you will look at 2 different bacteria, and you will use several different stains.

Procedure:

Work in pairs and divide the work between you, but make sure you look at all slides.

Negative staining

Negative staining uses a stain that is excluded from the cells, so you have a dark background with a light area highlighting each cell.

1. Mix a small drop of 4% nigrosine with a drop of Rhodospirillium suspension on a clean microscope slide.

2. Using a clean cover slip, smear the suspension over the slide, and allow it to dry completely under the lamp on the side bench.

3. Add one drop of immersion oil to the slide. This acts as a ‘mounting medium’, allowing you to now to cover your specimen with a coverslip.

4. Look at your cells under 10X to focus, and then under 40X. When you have a clear focus, add one drop of immersion oil on top of your coverslip, then examine the slide under 100X. Make notes and/or drawings of what you see.

What shape are the bacterial cells?

Positive staining

Positive staining stains cells, leaving the background unstained. Toluidine blue is a positive stain.

5. Put a drop of bacterial suspension on a slide, smear it as before, and allow it to dry under the lamp.

6. Add one drop of toluidine blue solution on top of your smear, and leave it for 5 minutes on the bench.

7. Gently rinse the slide with distilled water, using the squeeze bottles provided. Be careful not to squirt too hard, or you may wash the bacteria off your slide. Dry the back of your slide with a tissue, then leave it under the lamp to dry completely.

8. When the slide is dry, place a drop of immersion oil on the smear as a mounting medium, and cover with a coverslip.
9. Focus the bacteria clearly under the 40X objective, then add a drop of immersion oil on top of the coverslip, and examine under the 100X objective.

**What features can you see?**

**How does this stain compare with the nigrosine?**

**What shape are the bacteria?**

10. Report your findings with a drawing, including a scale (Figure 5)

Figure 5:
5. STAINING FIXED CELLS

Using stains directly on cells can be useful, but often, much more detail is visible if you fix your cells before staining. This is because fixing tends to make the membrane more permeable, and allow the stain to better enter the cell. Many fixatives are used, but a common one is ethanol. You are going to compare toluidine blue staining of fixed and unfixed cells of the cyanobacterium, *Anabaena*.

Unfixed cells:

1. Mix one drop of toluidine blue with one drop of *Anabaena* suspension directly on a slide. Cover it with a coverslip, and examine it under 40X.

   **Can you see the cell wall? (It should look like a pinkish fringe around the cell)**

   Describe:

   **Does the toluidine blue stain anything inside the cells?**

   **Can you think of an explanation for this?**

Fixed cells:

2. Place one drop of *Anabaena* suspension on a watchglass, and add one drop of 50% ethanol.

3. Mix well and leave for 2-3 minutes, then transfer one drop of this mixture to a slide, and add one drop of toluidine blue. Mix and leave for 2-3 minutes to stain.

4. Transfer 1 drop of stained suspension to a new slide, cover it with a coverslip, and examine it under 40X. If you need to move to 100X, add a drop of immersion oil on top of the coverslip.

   **What are the main features you see?**

   **What are the main differences between this slide and the unfixed cells?**
Can you think of a reason for the differences?

5. Make a drawing of both fixed and unfixed cells. Label features that you can identify, and indicate size with a scale (Figure 6)

Figure 6:

Write a brief explanation of the difference between the two slides.
6. POST- LAB DISCUSSION: SCIENTIFIC METHOD
Hypothesis: What do you think is going on?

All good science starts from an idea that is then tested. These ideas are termed “hypotheses”. When you design an experiment, you should start by thinking about what idea you are trying to test. A hypothesis can be very simple: for example you might start with an hypothesis “that all living things are made up of cells”. Of course such an hypothesis can be hard to prove (how could you test all living things?) but easy to disprove (if you find even one kind of living thing that is not made of cells). This is generally true of most hypotheses: they can be disproved but not proved. However, if you do lots of experiments that support an hypothesis, and cannot disprove it, the hypothesis gains strength and reliability.

Experimental design:

The foundation of any scientific investigation is its experimental design, a logical outline that guides the gathering and evaluation of information. It is the researcher's plan for testing the validity of a hypothesis. Much thought and hard work accompany the development of a hypothesis and experiments that will yield clear results. A scientist's ability to ask key questions and to formulate them into testable hypotheses may largely determine the success or failure of a given research project.

The very nature of some questions requires estimations or assumptions to be made. Whenever estimations or assumptions are part of the experimental design, they must be clearly stated and justified as part of the experiment. Any estimation is based on some type of data. The data must be shown as well as the calculations that lead to the final estimate. An assumption is based on some fact. Any assumptions that are key to the experimental design must be stated as well as the reason for starting with this assumption.

Three factors are essential for good experimental design:

- Proper controls must be incorporated into each experiment. A control group receives the same treatment as the experimental group except that the factor being tested is applied to the experimental group only, not to the control.

- Experiments must be repeated enough times to allow comparisons between experimental and control groups. It is through such repetition that data can be compared statistically and a high degree of accuracy obtained.

- Experiments must be designed to avoid bias. A researcher must strive to prevent personal opinion about a hypothesis from influencing how tests are made and must also be aware of the bias that any technique or instrument may introduce in the outcome of an experiment.
Performing the experiment:

Researchers are meticulous note-takers. They make detailed notes in a record book that becomes a scientific diary of the research project in process. Data, or results, accumulate as tests outlined in the experimental design are completed. There are two types of data:

I. Anecdotal data - relate in words what happens in an experiment, recording chance observations as well as describing mistakes and unexpected events.

II. Numerical data - consists of measurements determined by a person or instrument.

Collecting data can be a time-consuming, tedious process, and patience is an essential ingredient in science. As results accumulate, the researcher tries to find patterns or relationships in the data. Interpreting data by asking critical questions is essential to determining the cause and effect of experimental observations. When the results of repeated tests are consistent and patterns become discernible, the next stage of the process is reporting the results.

Reporting experimental results:

Science is a powerful group activity, providing many opportunities for correcting errors. Researchers formulate their ideas from data analysis, then describe these ideas at seminars and meetings pertaining to their particular fields. It is through presentations like these that the researcher has an opportunity to interact with others in the field and to see how well their work stands up to peer scrutiny. The researcher then decides whether more experiments are needed or whether it is time to publish the results. Publishing a scientific paper is the next step in the reporting process. Hundreds of professional societies throughout the world publish journals containing articles that describe original research. Work so published is then permanently available to the scientific community. Monitoring current developments and searching literature for pertinent information are ongoing aspects of any scientist's work. Most scientists subscribe to several journals and use abstracting services to find pertinent reports in their filed.

7. SELF-MANAGEMENT: ASSESSMENT REFLECTION

Research suggests that visualising and contrasting increase goal achievement.

Having now completed a BABS1201 assessment, ask yourself these questions:

Were you happy with what your preparation / performance?

What could you have done differently to prepare / perform better?

Did you manage your time adequately to complete the task? Did you use the self-management resources in the Course Manual / on Moodle? Did you go through all the steps illustrated in the goal selection video?
What obstacles existed, and how might you deal with them better next time?

Your tutor may take a look at your responses to see how they can assist you and direct you to helpful resources.

What is your next assessment task and when is it due?

1. How well do you want to do in your next assessment? (e.g. Pass, Distinction)

2. Spend a few minutes visualising, in detail, how it would be, to do well in this assessment. (e.g. feelings, what people would say when you tell them).

3. Then write down 3 benefits of achieving what you want in the next assessment.

4. In terms of where you are now, write down three obstacles that are currently in the way of moving toward to achieving your goal.

5. Now do a bit of brainstorming about how you might deal with those obstacles (e.g. the obstacle might be that you are a bit stuck about some aspects of revision - will you ask your tutor or convenor about this? e.g. is it the case that you have other assessments and you feel as though you do not have much time - will you use some of the tools, such as the self-management activities, to help you with this?)

6. Write down at least one thing that you will do TODAY toward achieving your goal with your next assessment be specific, use scheduled tasks at a given time and date. (e.g. “After dinner tonight at 8pm, I’m going to do a first draft of my protocol, so I know what additional research and skills I need to do a good job”).

Return to Step 5 - it may take some time and energy, and discussion with others, to continue to successfully come up with solutions to obstacles. But there will never be a perfect solution, and you have to start somewhere!

Return to Step 6 - research has also shown that writing down the sub-tasks necessary to achieve your goal will help motivate you to act on those sub-tasks.
SECTION 2: EXPLORING CELL FUNCTION
PRACTICALS 4 - 6

Over the next three practical classes, your mission will be to perform accurate and repeatable measurements of cell functions including:

- Osmosis and diffusion
- Enzyme function
- Photosynthesis and respiration

One of the crucial qualities expected of a scientist is to be able to accurately and reproducibly measure a particular parameter of cell function in order to draw conclusions and make new hypotheses, and it is upon this skill that these practicals are focussed.

The goals for this sequence are:

- To become proficient in the use of modern pipettors to:
  1. Accurately dispense a known volume of solution.
  2. Accurately dilute a concentrated solution to give different final concentrations.

- To accurately calculate results using appropriate formulae.

- To be able to design a basic experiment to test a desired hypothesis.

- To be able to use modern laboratory equipment to derive results from experiments.

- To communicate experimental results to other scientists.

Graduate attributes

- Research, inquiry and analytical thinking abilities
- Communication
- Information literacy
- Teamwork, collaborative and management skills
## Practical 4: Osmosis and Diffusion

### Student Risk Assessment

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<thead>
<tr>
<th>Hazards</th>
<th>Risks</th>
<th>Controls</th>
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<tbody>
<tr>
<td><strong>Physical injury (glass capillary tube)</strong></td>
<td>Cuts.</td>
<td>Do not bend the capillary tube. <strong>Hold the tube close to the end being inserted</strong> through the bung and take care to apply force only along the axis of the capillary tube.</td>
</tr>
<tr>
<td><strong>Hazardous chemicals (Congo Red)</strong></td>
<td><strong>Warning!</strong> Causes eye irritation. May cause skin and respiratory tract irritation. Possible risk of harm to the unborn child. <strong>May cause cancer in humans.</strong> May cause central nervous system effects. Ingestion: May be harmful if swallowed. Skin: May be harmful if absorbed through skin. May cause skin irritation. Eyes: May cause eye irritation.</td>
<td>Wear Personal protective equipment (gowns, gloves and enclosed shoes). Wash hands before leaving the laboratory. <strong>Wear gloves at all times when working with Congo Red.</strong> Do not use personal items such as mobile phones or computers with gloves on.</td>
</tr>
<tr>
<td><strong>Biological hazard (horse red blood cells)</strong></td>
<td>Infection: contains animal blood product.</td>
<td>Handle Material as potentially infectious.</td>
</tr>
</tbody>
</table>

### Personal Protective Equipment
- Closed in Footwear
- Lab. Coat
- Gloves
- Safety glasses

### Emergency Procedures
- In the event of an alarm sounding, stop the experiment, turn off electrical equipment and wait for confirmation from demonstrators to evacuate. Then pack up your bags and wash your hands. Follow the instructions of the demonstrators regarding exits and assembly points. The BioScience assembly point is in the lawn in front of the Chancellery. In the event of an injury inform the demonstrator. First aiders and contact details are on display by the lifts.

### Clean up and Waste Disposal
- All used gloves should be placed in the biohazard bins. Used test tubes should be placed in the appropriate container on the front bench of the lab. **All solutions should be placed in the appropriate liquid waste containers.** Used slides and cover slips should be placed in approved biohazard sharps containers.

### Declaration
- I have read and understand the safety requirements for this practical class and I will observe these requirements.

**Signature:** ................................................................. **Date:** ........................................

**Student number:** ........................................
WEEK 5
PRACTICAL 4
CELL FUNCTION I
OSMOSIS AND DIFFUSION

ASSESSMENT

Your scientific literature essay is due today. A hard copy of your essay must be submitted to your laboratory demonstrator at the commencement of today’s practical class. A copy must also be submitted to Turnitin on Moodle.

OBJECTIVES

- To calculate and report on parameters involved in diffusion through a semi-permeable membrane
- To investigate and report on the effects of osmosis on animal and plant cells

CONTENTS

1. Osmosis
2. Effect of osmosis on animal and plant cells
3. Post-lab discussion - Accuracy, precision & reproducibility
4. Discussion
5. Reflecting on mid-session preparation

1. OSMOSIS

Introduction:

Movement of substances through cell membranes can, at one extreme, be entirely dependent on physical factors (passive transport) or at the other extreme, may be entirely dependent on specific transport mechanisms that require energy for their function (active transport).

One of the most important factors influencing the passive movement of substances through cell membranes is membrane permeability. All cells are enclosed by a plasma membrane which is semipermeable. To be more accurate, the plasma membrane is selectively or differentially permeable to various solutes. Osmosis is the spontaneous net movement of water across such a semi-permeable membrane from a region of low solute concentration to one with a high solute concentration, down a solute concentration gradient. These descriptions all imply that the cell membrane is much more permeable to water than it is to most solutes dissolved in the water.

The net movement of a solvent (substance, usually a liquid, in which other substances are dissolved) is from the hypotonic (less-concentrated) to a hypertonic (more-concentrated) solution. This results in a reduced difference between the concentrations.
Experimental Procedure:

NOTE: Congo red will stain skin and clothing indelibly.

Work in pairs

In this part of the experiment, you will demonstrate osmosis using an artificial semi-permeable membrane, and calculate the osmotic potential. The osmotic potential of a solution that is separated from another solution by a semi-permeable membrane is a measure of potential of the solution to suck water across the membrane.

1. Take a 10 cm length of dialysis (cellophane) tubing. Wet the ends of the tubing. Insert a solid rubber bung into one end of the tubing and a perforated rubber bung into the other end. Wrap one or two rubber bands tightly around each of the rubber stoppers to make a leak-proof seal at each end of the dialysis tubing.

2. Over the sink, carefully fill the bag through the hole in one of the bungs with 3.5% w/v Congo red solution (i.e. 3.5 gm Congo red in 100 ml aqueous solution).

3. The molecular weight of Congo Red is approximately 700. What is the molarity of the Congo Red solution?

3. Insert the capillary tube into the hole in the bung until the red solution appears at the bottom of the tube above the bung. Do not bend the capillary tube. Hold the tube close to the end being inserted through the bung and take care to apply force only along the axis of the capillary tube. Wash the outside of the filled dialysis tube with water to remove any spilled Congo red solution.

4. Examine the apparatus for leaks.

5. Support the capillary tube on a retort stand so that the capillary tube is vertical and the dialysis bag is completely immersed in a beaker of distilled water, as illustrated in Figure 1 on the following page.
6. Note the level of the Congo red solution in the capillary tube.

Measure the level every 20 minutes for the next 2 hours and plot the results against time on the blank graph provided as Figure 2 (don’t forget to give the figure a title).
8. Use Equation 1 below to calculate the osmotic pressure exerted by the congo red solution under these circumstances. Show your calculations.

**Equation 1:** \[ \Delta \pi = 1000 \, RT \, (C_i - C_o) \]

Where:
- \( \pi \) = osmotic pressure (N.m\(^{-2}\))
- \( R \) = universal gas constant (8.3 J mol\(^{-1}\) K\(^{-1}\))
- \( T \) = absolute temperature (Kelvin, note that 0 Kelvin = -273°C)
- \( C_i \) = concentration inside bag (mol.L\(^{-1}\))
- \( C_o \) = concentration outside bag (mol.L\(^{-1}\))

9. Use Equation 2 below to calculate the height of water that can theoretically be supported by this solution. Show your calculations.

**Equation 2:** \[ P = \rho \, gh \]

Where:
- \( \rho \) = density of water (1000 kg.m\(^{-3}\))
- \( P \) = hydrostatic pressure (N. m\(^{-2}\))
- \( g \) = acceleration of gravity (9.8 m.s\(^{-2}\))
- \( h \) = height (m)
Given sufficient time, would the liquid column in the capillary tube reach this height?

Explain your answer:

10. Look at the demonstration using 2% methyl blue as the osmotic agent instead of congo red. The molecular weight of this molecule is almost the same as congo red.

Can you think of a reason why Methyl blue readily escapes from the dialysis bag while Congo red does not?
2. EFFECT OF OSMOSIS ON ANIMAL AND PLANT CELLS

Two solutions containing different solutes but having the same osmotic pressure are called iso-osmotic. However, if these two solutions are separated by a membrane they may not exert the same osmotic pressure across the membrane. This will depend on the permeability of the membrane to each of the two solutes. When two such solutions do exert the same osmotic pressure in a membrane system they are described as being isotonic. Osmotic potential depends on solute concentration and temperature whereas tonicity depends on solute concentration, temperature and the relative permeability of the membrane to the solutes.

In the next exercise, the effect of osmotic gradients on animal and plant cells will be demonstrated by lysis (bursting) of animal erythrocytes (red blood cells) and by plasmolysis (cytoplasmic shrinkage) of plant cells.

Animal erythrocytes:

1. To measure the tonicity of animal erythrocytes (red blood cells) you will set up a series of salt solutions of differing tonicities, and you will see whether the erythrocytes lyse or not.

2. Set up a series of 10 labelled test tubes in a test tube rack.

3. Use the pipettor to dilute the stock 0.2 M (200 Mm) sodium chloride (NaCl) provided with distilled water to make up the range of solutions given in Table 1. Your tutor will demonstrate the correct use of the pipettor.

Table 1. Concentrations of Sodium Chloride (NaCl)

<table>
<thead>
<tr>
<th>Tube</th>
<th>Volume of NaCl (Ml)</th>
<th>Volume of dist. Water (Ml)</th>
<th>Final NaCl conc. (Mm)</th>
<th>Result (lysis or no lysis)</th>
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<tbody>
<tr>
<td>1</td>
<td>1.25</td>
<td>3.75</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.50</td>
<td>3.50</td>
<td>60</td>
<td></td>
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<tr>
<td>3</td>
<td>1.75</td>
<td>3.25</td>
<td>70</td>
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<td>4</td>
<td>2.00</td>
<td>3.00</td>
<td>80</td>
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<td>5</td>
<td>2.25</td>
<td>2.75</td>
<td>90</td>
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<td>6</td>
<td>2.50</td>
<td>2.50</td>
<td>100</td>
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<td>7</td>
<td>2.75</td>
<td>2.25</td>
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<td>8</td>
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<td>9</td>
<td>3.25</td>
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<td>3.50</td>
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<td>140</td>
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4. To each tube, add 0.1 ml sheep blood and shake well.

5. Let the tubes stand undisturbed in the rack for at least 1 hour to allow the non-lysed cells to settle.

6. If the erythrocytes are not lysed, the tube will appear cloudy and red at the bottom where the cells are, and clear at the top.

7. If the blood has lysed at all, the supernatant (above the cells) will begin to be tinted red from the haemoglobin that has escaped from the burst cells.

8. With complete lysis, the tube will appear clear and red.

9. Place a piece of white paper behind the tubes, and identify which tubes show each of the above features. Record your observation in the RESULTS column of Table 1.

What is your estimate of the tonicity of the erythrocytes? Explain your answer:

Solute permeability

In this experiment, you will investigate the effect of solute permeability into erythrocytes on the outcome of the lysis process, remembering that unless the solute can penetrate the erythrocyte membrane, osmosis cannot occur. All the solutions you will use are 330 Mm. A range of different solutions, including sucrose, glucose, urea, glycerol and ethyl alcohol are provided.

Decide what factors might affect the permeability of different solutes into erythrocytes. Explain your reasoning:

10. Choose two solutions from those available, making sure you choose one that you think will easily penetrate the cells, and one which will not. Make sure that at least one person within your bench group will be testing every solution.

11. Place 2 Ml of each of your solutions into separate small test tubes. Add 2 drops of blood to each tube and quickly stir.
12. Using a stop watch if necessary, note the time taken for complete lysis of the 
erthrocytes. Keep checking your tubes at 1 minute intervals for the first 10 
minutes, then 5 minute intervals for one hour if necessary.

13. Record the time taken for lysis in each of your tubes. Consult with the other 
members of your bench group, and complete the table of the time taken for lysis 
upon exposure to each solution (Table 2).

Table 2: Estimation of induced lysis of erythrocytes

<table>
<thead>
<tr>
<th>Solution</th>
<th>Time until complete lysis of erythrocytes</th>
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Were the erythrocytes affected by the different solutions in the ways that you 
predicted? If this was not the case, can you suggest reasons for this?
Estimation of osmotic concentration of plant cells:

When plant cells are immersed in a hypotonic solution, the large vacuoles of the cells swell until the positive hydrostatic pressure has increased to balance the negative osmotic potential.

Why doesn’t the cell burst like an animal cell?

The average osmotic concentration of the intracellular fluid of the population of plant cells can be estimated using the phenomenon of plasmolysis.

Plasmolysis occurs when the osmotic potential gradient is reversed by placing cells in a hypertonic solution. Water then diffuses out of the vacuole into the external solution. Incipient plasmolysis describes the condition when a solution removes sufficient water to cause the protoplast to detach from the cell wall.

The concentration that causes plasmolysis in 50% of the cells can be estimated by placing pieces of plant tissue into a graded series of solutions. This concentration is isotonic with the vacuolar contents of an average cell in the tissue.

You should work as a bench group for this experiment. Each student should perform counts on at least two tubes.

Students are advised to put gloves on when handling Rhoeo because the plant can be an irritant.

Procedure:
1. Cut 9 thin slices from the purple epidermis on the underside of the Rhoeo leaf. Note: any slice that has numerous green photosynthetic cells attached to its under-surface is too thick. Immerse the slices in distilled water as they are cut.

2. Set up a series of 8-10 labelled specimen tubes.

3. You will now use the pipettor to dilute a 0.5 M (500 Mm) NaCl solution with distilled water to a range of concentrations from 500 Mm down. Make sure you cover the range well so you can narrow down the concentration that is isotonic with the plant vacuole.

4. You need a total volume of 10 Ml in each tube, so use the table below to help you calculate the relative volumes of NaCl and water you need for each concentration. For example, if you wanted to test NaCl at 500 Mm, you would add 10 Ml NaCl solution and 0 Ml distilled water. If you wanted to test 250 Mm, you would add 5 Ml NaCl solution plus 5 Ml distilled water. Complete Table 3, to indicate the range of concentrations you will test.
Table 3: Concentrations of NaCl for testing plasmolysis

<table>
<thead>
<tr>
<th>Tube</th>
<th>Volume of NaCl (mL)</th>
<th>Volume of distilled water (mL)</th>
<th>Final NaCl concentration (mM)</th>
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Show your table to your demonstrator. While there is no one correct answer for the appropriate concentrations of NaCl to use, your tutor will review your calculations for the dilutions.

5. Before placing any pieces of plant epidermis into the tubes, mount one slice in distilled water on a microscope slide with the cuticle uppermost, and cover with a cover-slip.

6. Select two tubes from your series, one from the highest half of the dilutions series, one from the lower half, e.g. you might choose tubes 1 and 5, or 2 and 6 etc.

7. At 3 minute intervals, totally immerse one of the slices of epidermis in one of your tubes, and leave for 20 minutes.

8. After 20 minutes, remove each piece, and mount it in the solution in which it was soaking on a microscope slide with the cuticle uppermost, and cover it with a cover-slip.

9. Immediately examine at least 50 cells for plasmolysis. Any cell showing visible separation of the purple protoplast from the cell wall should be counted as plasmolysed.

10. Record the total number of cells you count, and the number of these that are plasmolysed. From this, calculate the percent plasmolysed at each NaCl concentration.
Obtain the results from other members of your group, and record all in Table 4.

Table 4: Results

<table>
<thead>
<tr>
<th>Tube</th>
<th>NaCl concentration (mM)</th>
<th>Total no. cells counted</th>
<th>No. of cells plasmolysed</th>
<th>% cells plasmolysed</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>
Plot the % plasmolysis versus NaCl concentration as Figure 3 below.
What concentration do you estimate to be isotonic with your plant cells?

Would the intracellular fluid of Rhoeo be isotonic, hypertonic or hypotonic with respect to the sheep erythrocytes you investigated earlier?

What occupies the space between the plasmolysed protoplast and the cell wall of the Rhoeo cells?

3. POST-LAB DISCUSSION:
   ANALYSING DATA - ACCURACY, PRECISION & REPRODUCIBILITY

Reproducibility and accuracy are the essence of science. Your data needs to be:

- **Reliable/reproducible**: if you repeat the measurement several times under the same conditions you should get the same answer.

- **Accurate**: your measurement should give you the correct value or very close to it.

An unreliable method cannot be accurate, but reproducibility does not guarantee accuracy.

*Can you think of an occasion where a reproducible measurement might not be accurate?*

**Activity**: Your demonstrator will discuss today’s activity.
4. REFLECTING ON MID-SESSION & ESSAY PREPARATION

Think about your preparedness for you assessments and how it relates to your academic goals. How on track are you? (mark appropriate place on the line):

0%____________________________________________________________ 100%

How might you improve your rating next week?

Write down what you can do TODAY towards your academic tasks/sub-goals TOMORROW:

Write down what you can do THIS WEEK towards your academic tasks/sub-goals NEXT WEEK:

Encourage yourself by writing down what you have completed/achieved:

On the following scale, please indicate how much time and effort you dedicated to each of the following domains of life in the past week. YOU be the judge of how much you need of each.

<table>
<thead>
<tr>
<th></th>
<th>not enough</th>
<th>enough</th>
<th>a lot</th>
<th>too much</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleeping</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Studying</td>
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<tr>
<td>Socialising</td>
<td></td>
<td></td>
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<tr>
<td>Exercising</td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Reflection Questions

Have I been studying enough or have I been procrastinating?

How was this week different from last week, is the change positive?

What is ONE domain where you want to do LESS next week?

What is ONE domain where you want to do MORE next week?

While studying or thinking about studying, I have been (circle):

  anxious   stressed   distracted   focused/present

When thinking about my ability to manage my workload, I have felt:

  hopeless   pessimistic   neutral   optimistic   confident

If you find yourself on the left side of either of the last two scales, consider a visit to The Hub: [https://student.unsw.edu.au/hub](https://student.unsw.edu.au/hub)

Your tutor may take a look at your responses to see how they can assist you and direct you to helpful resources.
WEEK 6

NO PRACTICAL CLASS

No practical classes this week due to the Good Friday Public Holiday
### General

<table>
<thead>
<tr>
<th>See below</th>
</tr>
</thead>
</table>

**At all times** follow demonstrators instructions when handling all biological substances, chemicals and equipment items. Notify demonstrator immediately of any spills or other incidents. NO eating drinking or smoking in lab.

PPE (lab coats, closed in shoes & gloves as required), Dispose of all sharps in the sharps containers provided on your bench. Proper handwashing before leaving the laboratory.

### Physical injury (plastic capillary tube)

<table>
<thead>
<tr>
<th>Cuts.</th>
</tr>
</thead>
</table>

Breaking tube when inserting into rubber bung.

**Do not bend the capillary tube.**

**Hold the tube close to the end being inserted** through the bung and take care to apply force only along the axis of the capillary tube.

### Hazardous chemicals (soda lime)

<table>
<thead>
<tr>
<th>Warning! Soda lime is a strong alkali. Do not get in on skin or clothing. If you do, wash the affected area for several minutes with running water.</th>
</tr>
</thead>
</table>

**Wear Personal protective equipment (gowns, gloves and enclosed shoes).** Wash hands before leaving the laboratory.

**Wear gloves at all times.**

---

**In the event of an alarm sounding, stop the experiment, turn of electrical equipment and wait for confirmation from demonstrators to evacuate. Then pack up your bags and wash your hands. Follow the instructions of the demonstrators regarding exits and assembly points. The BioScience assembly point is in the lawn in front of the Chancellery. In the event of an injury inform the demonstrator. First aiders and contact details are on display by the lifts.**

All used gloves should be placed in the biohazard bins. Used test tubes should be placed in the appropriate container on the front bench of the lab. **All solutions should be placed in the appropriate liquid waste containers.** Used slides and cover slips should be placed in approved biohazard sharps containers.

I have read and understand the safety requirements for this practical class and I will observe these requirements.

**Signature:**…………………………………………………………………………………………………………………………

**Date:**………………………………………………………………………………………………………………

**Student number:**………………………………………………………………………………………………………………

---
ASSESSMENT

Your group enzymes project experimental protocol is due today. A hard copy of your group’s protocol (based on the template provided via Moodle), signed by all members of the group, must be submitted to your laboratory demonstrator at the commencement of today’s practical class.

Meet with your Biological Modelling group for 5 minutes and use the template on Moodle to schedule and plan your first/next meeting.

MASTERING BIOLOGY

This week there are three short tutorial activities for you to complete. You may begin these while you wait for the class to start. The activities will help you prepare for your Mastering Biology Quiz 3 Photosynthesis which you must complete by the end of Week 9.

Objectives

- To investigate the effect of light intensity on the rate of photosynthesis
- To determine the rate of respiration in mung beans and the effects of different metabolic inhibitors on that rate.

Contents

1. Photosynthesis:
   1a. Rate of photosynthesis and light intensity in a whole plant
   1b. Structure of a chloroplast
   1c. The Hill reaction
2. Respiration
3. Post-lab discussion
1. PHOTOSYNTHESIS

Introduction:
Photosynthesis is frequently defined as the process by which green plants manufacture carbohydrates from carbon dioxide and water, using radiation from the sun as a source of energy. The overall process is summarised by the equation:

\[ 6\text{CO}_2 + 6\text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \]

Photosynthesis is not a single one-step process as this equation implies, but a complex series of chemical reactions. The overall process is divided into two quite separate parts.

The so-called light reactions are a series of photochemical reactions that take place on the thylakoid membranes of the chloroplast. These reactions are also called the Hill reactions. In these reactions:

- The energy provided by light splits a water molecule
- Gaseous oxygen is liberated as a result
- The energy from the light is trapped in the short-term chemical intermediaries ATP and NADPH

The so-called dark reactions are reactions in which carbohydrates are made from carbon dioxide by using the reducing power of the ATP and NADPH generated in the light reactions. Since production of carbohydrates can occur with or without light, it is perhaps misleading to call it the dark reaction, and the process is better termed the Calvin cycle. Only the light reactions are unique to photosynthesis. You will look at this part of the process today.

1a RATE OF PHOTOSYNTHESIS & LIGHT INTENSITY IN A WHOLE PLANT

Demonstration:
This experiment using the aquatic plant *Egeria densa* has been set up as a demonstration. The rate of photosynthesis at different light intensities was measured by the rate of oxygen production, as indicated by the rate of bubbling. Other factors that might affect the photosynthetic rate were kept constant. The data are set out with the demonstration. These light measurements were made on the axis of the lamp, in the centre of the beam. They do not follow the inverse square law because the lamp and reflector are not a point source over these distances.
Why is the relationship between photosynthetic rate and light intensity curvilinear with a tendency to saturate at higher light intensities?
1b  STRUCTURE OF A CHLOROPLAST

Examine the electron micrograph of a chloroplast.

Identify and label the following components of a chloroplast (Figure 1).

- The inner and outer membranes
- The thylakoid membrane stacks (grana)
- The stroma
- The location of the Hill reaction (light reactions) and the Calvin cycle (dark reactions).

Figure 1: Chloroplast.

1c.  THE HILL REACTION

In 1939, Robin Hill of Cambridge University found that suspensions of chloroplasts isolated from leaf cells yielded oxygen when illuminated in the presence of an electron acceptor, that is, an oxidising agent. Hill demonstrated the essential features of the light reactions. These reactions are summarised in Figure 2.

Figure 2. Summary of the light reactions.
Since the oxidising agent used by plants in intact chloroplasts is \( \text{NADP}^+ \), the light reaction can be written as:

\[
2\text{H}_2\text{O} + 3\text{ADP} + 3\text{P} + 2\text{NADP}^+ \rightarrow \text{O}_2 + 2\text{NADPH} + 3\text{ATP} + 2\text{H}^+
\]

Hill used an artificial oxidising agent, ferric cyanide. Modern versions of the reaction use an oxidising agent called dichlorophenolindophenol (DPIP for short) in place of NADP. So the Hill reaction can be written as:

\[
2\text{H}_2\text{O} + 2\text{DPIP}^{\text{light}} \rightarrow 2\text{DPIPH}^{\text{*}} + \text{O}_2 \text{ (in the presence of chloroplasts)}
\]

*Oxidised DPIP is blue  
**Reduced DPIP is colourless.

Therefore, the rate of disappearance of the blue DPIP indicates the rate of the Hill reaction.

No ATP is made in the Hill reaction because the method of preparation partially ruptures the chloroplast membranes. Thus, the gradient of \( \text{H}^+ \) concentration, which drives ATP synthesis, cannot form.

The Hill reaction was the first demonstration of an \textit{in vitro} (literally, in glass, that is a test-tube) reaction similar to the photosynthetic activity of the chloroplasts during \textit{in vivo} (literally, in life) photosynthesis.

\textbf{Aim:}

The aim of this experiment is to demonstrate the transfer of electrons from chlorophyll \( a \) to a suitable electron acceptor and the subsequent replacement of those electrons from water molecules so releasing \( \text{O}_2 \).

\textbf{Preparing the chloroplasts:}

1. Work in groups of 3. Take 5 g of spinach leaf. Remove the main vein and grind up the leaf tissue with a 1/4 teaspoon of sand and 3 mL of buffer-sucrose solution. Grind thoroughly keeping the mortar and pestle on ice. When the visible lumps have disintegrated, add a further 11 mL of buffer to the homogenate.

2. Put a 100 mL beaker in an ice bucket to act as a receptacle and strain the homogenate through two layers of damp cheese-cloth into the chilled beaker. Squeeze the cheese-cloth out to make sure that all liquid is extracted into the beaker.

3. Take a larger test tube and mark it at a level equivalent to 30 mL volume. Pour the mixture from the beaker into the large test tube and add buffer to make the volume up to the 30 mL mark. Keep the mixture on ice. Stir the mixture before taking an aliquot for the experiment.
Trial run:

1. Wrap a thin sheet of foil around each of two test tubes. Leave them in a test tube rack. Label the tubes A and B. To A add 6 mL of buffered sucrose solution and 0.5 mL of 2,6 dichlorophenolindophenol (DPIP, strength 0.4 mmol.L\(^{-1}\)). To tube B add 6.5 mL of buffered sucrose solution.

2. Half-fill a beaker to act as a water bath. Place the test tube holder in the beaker, with 2 empty tubes (the rubber rings are for the bottom of the tubes, so they stay upright).

3. Direct a 240 V 60 W bench lamp onto the tubes at about 10 cm distance from them (that is, 10 cm from the rim of the lamp holder to the test tubes), but keep the light turned off. Check that your light is in a direct line with the tubes.

4. Add 0.5 mL of your chloroplast suspension to each of tubes A & B and stir the mixture. Quickly remove the foil from tubes A and B. Remove the empty tubes from the holder in the H\(_2\)O bath and replace with tubes A and B.

5. Turn on the light. Start the timer and time the disappearance of the blue colour from the tube containing DPIP (e.g. when the tube containing DPIP is the same green colour as the control tube).

Which reaction leads to this change in colour?

Effect of light intensity:

Repeat the above experiment with the bench lamp at 15, 20, 40 and 60 cm from the test tubes.

From the standard curve provided on the side bench, calculate the light intensities at each distance. Enter all your results in the table below. The constant initial concentration of DPIP has been entered for you.

<table>
<thead>
<tr>
<th>Table 1.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance (cm)</td>
</tr>
<tr>
<td>Light intensity</td>
</tr>
<tr>
<td>A. Initial DPIP (nmol)</td>
</tr>
<tr>
<td>B. time for decolourisation (sec)</td>
</tr>
<tr>
<td>C. Rate of breakdown (A/B)</td>
</tr>
</tbody>
</table>
Express your results as a graph (Figure 3). Make sure your graph is fully labelled and accurately represents the data in the table.

**What can you conclude from this experiment?**

**What is the role of tube B? Is it effective in this role? If not, what would you suggest in its place?**
2. RESPIRATION

Introduction:

In its broadest meaning, respiration means the release of energy from complex organic molecules built up during the process of photosynthesis. The overall process can be summarised as:

\[
\text{Glucose + oxygen } \xrightarrow{\text{enzymes}} \text{ energy} \rightarrow \text{ ATP, lost as heat, carbon dioxide + water}
\]

Thus, respiration reverses the action of photosynthesis by liberating the chemical energy stored in the glucose molecule. However, respiration is not simply a reversal of the reaction sequence of photosynthesis.

There are several ways in which we can demonstrate that respiration is occurring in an organism:

- by measuring the energy given off in the form of heat,
- by measuring the amount of glucose used,
- by determining the amount of oxygen consumed,
- by measuring the amount of carbon dioxide given off.

Today you will measure respiration in beans by measuring oxygen consumption.

The experiment will be performed with a respirometer. Changes in the volume of gas in the respirometer are shown by the movement of coloured fluid in a plastic tube connected to a container.

Procedure:

<table>
<thead>
<tr>
<th>Safety Warning:</th>
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<tbody>
<tr>
<td>Soda lime is a strong alkali. Do not get in on skin or clothing. If you do, wash the affected area for several minutes with running water.</td>
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</tbody>
</table>

Work in groups of 3.

Fill a test tube with germinated mung beans.
1. Put soda lime in the small wire basket and place it in the test tube, making sure that it is not touching the beans.

2. Using the Figures 4 and 5 as a guide, construct your respirometer as follows:

Figure 4: Respirometer design.

Figure 5: Respirometer design.
3. Dampen the sides of the rubber bung with water and insert it with its plastic tube firmly into the test tube.

4. Attach the pinch clamp to the rubber tubing, but do not clamp it yet. A millimetre scale is firmly attached to the plastic tube.

5. Set up the respirometer in a horizontal position on the stand.

6. Affix the flat-bottomed tube containing the dye solution to the other end of the plastic tubing so that the end of the plastic tubing is about one centimetre from the bottom of the tube containing the dye.

7. Keep your respirometer away from heat sources, as it is very sensitive to heat.

8. Allow your experiment to equilibrate for about 5 minutes, and then tighten the clamp.

9. Wait several minutes until the end of the dye column reaches the millimetre scale. Take the initial reading for your experiment.

10. Over the next 45 minutes, take readings of the location of the liquid at 5 minute intervals. Compare your results with the demonstration of sterilised beans set up in the laboratory.

11. At the end of your experiment, the dye column can be returned to the flat-bottomed reservoir container by opening the pinch clamp and tilting the plastic tube. Blow any droplets of dye solution remaining in the plastic tube onto a piece of paper towel using compressed air from the tap at the front of the laboratory.

12. Analyse your results as follows:

13. The internal diameter of the plastic tubing is 3.0 mm. Use the formula for the volume of a cylinder to calculate the volume of gas consumed in the tube at each time point. Record your results in Table 5.

14. Plot a graph (Figure 6) of your experimental results on the graph provided over the page, showing the cumulative volume of gas consumed in the tube at three minute intervals. Make sure that your graph is well labelled and that the data correspond to those in your table.

15. Compare your results with those of other groups.

How reproducible are your results? How can you evaluate this?
Why is it necessary to put soda lime into the respirometer?

What is the purpose of the sterilized mung beans? Can you think of an alternative way of doing this?

Was there a difference in the oxygen consumption of the fresh and sterilised mung beans? If so, can you explain this?

Did you observe water vapour within the respirometer? Where may this water have come from?
Table 2.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Height of liquid (mm)</th>
<th>Volume of gas consumed (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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</table>
WEEK 8

NO PRACTICAL CLASS

No practical classes this week due to the ANZAC DAY Public Holiday
### General

<table>
<thead>
<tr>
<th>Hazards</th>
<th>Risks</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical injury (scalpels, knives, razor blades, hot water baths)</td>
<td>Cuts and burns</td>
<td>At all times follow demonstrators instructions when handling all biological substances, chemicals and equipment items. Notify demonstrator immediately of any spills or other incidents. NO eating drinking or smoking in lab. PPE (lab coats, closed in shoes &amp; gloves as required), Dispose of all sharps in the sharps containers provided on your bench. Proper handwashing before leaving the laboratory.</td>
</tr>
<tr>
<td>Hazardous chemicals (acids, bases, pH buffer solutions, hydrogen peroxide solution, iodine solution)</td>
<td>Do not get in on skin or clothing. If you do, wash the affected area for several minutes with running water.</td>
<td>Wear Personal protective equipment (gowns, gloves and enclosed shoes). Wash hands before leaving the laboratory. Wear safety glasses when working with concentrated acids, bases or hydrogen peroxide.</td>
</tr>
</tbody>
</table>

**Emergency Procedures**

In the event of an alarm sounding, stop the experiment, turn of electrical equipment and wait for confirmation from demonstrators to evacuate. Then pack up your bags and wash your hands. Follow the instructions of the demonstrators regarding exits and assembly points. The BioScience assembly point is in the lawn in front of the Chancellery. In the event of an injury inform the demonstrator. First aiders and contact details are on display by the lifts.

All used gloves should be placed in the biohazard bins. Used test tubes should be placed in the appropriate container on the front bench of the lab. **All solutions should be placed in the appropriate liquid waste containers.** Used slides and cover slips should be placed in approved biohazard sharps containers.

I have read and understand the safety requirements for this practical class and I will observe these requirements.

Signature: .......................................................... Date: ........................................

Student number: ........................................
WEEK 9
PRACTICAL 6
CELL FUNCTION III
ENZYMES

ASSESSMENT

Today you will be carrying out the experimental procedures designed by your project group to investigate enzyme activity. The experimental notes, results and observations that you record today will be checked and assessed by your demonstrator at the end of the practical (see below for details).

CONTENTS

1. Group Enzymes Project
2. Structuring a scientific report
3. Enzymes report guidelines and marking scheme

1. TEAM ENZYME PROJECT

After your demonstrator has shown your group where to locate all the necessary reagents and equipment required for your experiment, you can get started!

Use the following blank pages to write experimental notes, observations and raw data/results for your experiment. If more space is required, write on separate sheets of blank or lined paper and secure these to your manual, preferably using a stapler.

NOTE: All experimental notes, raw data and observations will be checked, signed and marked on an individual basis by your demonstrator at the end of this laboratory class. It is your responsibility to ensure that you have written down everything and that you are not relying on your other team member(s) to collect the information. You will also need to hand a copy these in with your report.
Team Enzyme Project: Experimental Notes, Observations and Results
Team Enzyme Project: Experimental Notes, Observations and Results

Demonstrator Signature:          Date:
TEAM ENZYME PROJECT – SCIENTIFIC REPORT

Please refer to pages 27-34 of this manual for general advice on how to construct a scientific report and specific report criteria with associated marking scheme. There is also a ‘Report Checklist’ on page 34 that you can use to ensure that you have satisfied the essential report requirements prior to its submission.

REPORT DUE DATE:

Remember that a hard copy of your report is due to be submitted to your DEMONSTRATOR at the BEGINNING of your Week 10 laboratory class. The penalty for late reports is 10% per day, including weekends.

Happy report writing!!!
SECTION 3:
EXPLORING GENES
PRACTICALS 7 - 9

Your mission in this section is to explore:

- The roles of genes and DNA in inheritance.
- The mechanisms by which DNA is passed from cell to cell when they divide.
- The use of molecular biology in disease diagnosis.

The third and final sequence of practical classes involves three labs exploring aspects of cell division, genetic inheritance, and molecular biology related to the concepts that will be discussed in lectures.

With the publishing of the human genome (all the genes in a human), and of multiple genomes of other living organisms including mice, dogs, horses, many plants, many bacteria and viruses, and many protists, modern genetics is one of the most important basic sciences supporting modern biological research. It is on some of the important concepts underlying genetics that this section is focussed.

The goals for this sequence are:

- To be able to explain the differences between mitosis and meiosis, and to recognise important stages in each
- To be able to reconstruct a human karyotype by organising metaphase chromosome spreads and to interpret your findings
- To analyse the inheritance of characteristics and explain the genetic basis of this
- To understand the principles of Polymerase Chain Reaction (PCR) and to interpret the results of a PCR experiment and construct a pedigree from those results.

Graduate attributes:

Your assignments for this section of the course can be included in your portfolio as evidence of the following Science Graduate Attributes:

- Research, inquiry and analytical thinking abilities
- Capability and motivation for intellectual development
- Information literacy
### Hazards

**General**
See below

**At all times** follow demonstrators instructions when handling all biological substances, chemicals and equipment items. Notify demonstrator immediately of any spills or other incidents.

<table>
<thead>
<tr>
<th>Physical injury (razor blades, needles)</th>
<th>Cuts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do not walk around laboratory with exposed sharps. Dispose of all used blades in the sharps bins provided on your bench.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hazardous chemicals (45% acetic acid mounting medium, 70% ethanol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do not get in on skin or clothing. If you do, wash the affected area for several minutes with running water.</td>
</tr>
<tr>
<td>Wear Personal protective equipment (gowns, gloves and enclosed shoes). Wear safety glasses when working with concentrated acid.</td>
</tr>
<tr>
<td><strong>Wear gloves at all times.</strong></td>
</tr>
</tbody>
</table>

---

### Emergency Procedures

In the event of an alarm sounding, stop the experiment, turn off electrical equipment and wait for confirmation from demonstrators to evacuate. Then pack up your bags and wash your hands. Follow the instructions of the demonstrators regarding exits and assembly points. The BioScience assembly point is in the lawn in front of the Chancellery. In the event of an injury inform the demonstrator. First aiders and contact details are on display by the lifts.

---

All used gloves should be placed in the biohazard bins. Used test tubes should be placed in the appropriate container on the front bench of the lab. **All solutions should be placed in the appropriate liquid waste containers.** Used razor blades, slides and cover slips should be placed in approved biohazard sharps containers.

---

I have read and understand the safety requirements for this practical class and I will observe these requirements.

Signature: ____________________________ Date: ____________________________

Student number: ____________________________
ASSESSMENT

Your individual scientific report on the Group Enzymes Project (15%) is due today. You must submit a hard copy of your report (with completed coversheet attached) directly to your demonstrator at the BEGINNING of today’s lab class.

MASTERING BIOLOGY TUTORIALS

This week there are three short tutorial activities for you to complete. These will help you to answer the questions for your Mastering Biology quiz 4 which you must complete by the end of Week 11.

Work through the following three BioFlix tutorials:

<table>
<thead>
<tr>
<th>ITEM TYPE</th>
<th>TITLE</th>
<th>MEDIAN TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tutorial</td>
<td>Meiosis (1 of 3): Genes, Chromosomes, and Sexual Reproduction (BioFlix tutorial)</td>
<td>13 min</td>
</tr>
<tr>
<td>Tutorial</td>
<td>Meiosis (2 of 3): The Mechanism (BioFlix tutorial)</td>
<td>10 min</td>
</tr>
<tr>
<td>Coaching Activities</td>
<td>Video Tutor Session Quiz: Mitosis vs. Meiosis</td>
<td>5 min</td>
</tr>
</tbody>
</table>

OBJECTIVES

- Describe human karyotyping and its role in ascertaining sex and detecting chromosomal abnormality.
- List and explain the principal events in mitosis and meiosis.
- Define the following terms: diploid, haploid, homologous chromosomes, locus, alleles.
- Explain the difference between the first and second meiotic divisions.
- List and explain the similarities and differences between meiosis and mitosis.
- Define the following terms: heterozygous, homozygous, dominant and recessive.

CONTENTS

1. Observing karyotypes
2. Mitosis in the onion root tip
3. Meiosis
4. Post - lab discussion
INTRODUCTION

The production of new cells continues throughout the life of any multicellular plant or animal. Unless there is some mishap, each cell divides to produce two exact genetic replicas of itself. This is the result of a process called mitosis, the division of the chromosomes. The chromosomes are located in the nucleus, and they contain the DNA, which carries the genetic information. The genes controlling a specific characteristic, for example, eye colour, are always at the same place ("locus") on a specific chromosome.

Understanding mitosis (and meiosis) is important for understanding how genetic information is passed from a cell to its daughter cells.

We will follow the behaviour of these chromosomes through a complete cycle of cell division. Although it is a continuous process, mitosis is divided into stages for convenience. These stages, which can be recognised down the microscope, are named as follows: prophase, metaphase, anaphase, telophase.

Successive mitotic divisions alternate with a much longer interphase. Diagrams and photographs of each stage are placed around the laboratory. For more detail on each of these phases, see the textbook.

![Figure 1: The cell cycle. G₁ is the first growth phase, and G₂ is the second growth phase.](image)
Human karyotype
A somatic human cell is diploid and usually contains 46 chromosomes, consisting of 23 homologous pairs. One of the homologous pairs are the sex chromosomes (XX in females or XY in males). The non-sex chromosomes are called autosomes. The karyotype of a species describes the chromosome complement of an organism in terms of chromosome number and length, centromere position and any other characteristics such as banding patterns seen with certain staining methods.

Many human hereditary defects caused by chromosomal abnormalities can be identified by examining human chromosomes from cells that have been arrested in metaphase of mitosis – a stage when chromosomes are very short and compact. Leukocytes (white blood cells) or fetal cells obtained by amniocentesis or chorionic villus sampling are often used for diagnosis.

The cells are cultured (to increase their number), treated with a chemical that disrupts the mitotic spindle (to stop mitosis), and placed in a hypotonic salt solution (to swell their nuclei). (Note: the mitotic spindle is a structure made of microtubules, which coordinates the movement of chromosomes during cell division). The mixture is then centrifuged (to increase the concentration of cells) and transferred to a glass slide. As a drop of the cell suspension hits the slide, the nuclei break open and the chromosomes spread apart; usually chromosomes from a single cell remain in an identifiable group. The cells are then stained using procedures that result in banded chromosomes.

In the early days of studying the human karyotype, it was hard to tell individual chromosomes apart. So they were classified into seven major groups, A through G. These groups were based on their length and the position of the centromere (the constricted point on the chromosome). The groups were:

- Group A: Chromosomes 1-3
- Group C: Chromosomes 6-12
- Group E: Chromosomes 16-18
- Group G: Chromosomes 21 and 22
- Group B: Chromosomes 4 and 5
- Group D: Chromosomes 13-15
- Group F: Chromosomes 19 and 20
- Sex chromosomes X and Y

Eventually, scientists could tell individual chromosomes apart thanks to coloured bands that were visible after staining (“painted chromosomes”).

Karyotype analysis involves a number of steps that enable visualisation of an individual’s chromosomes. First, cells from the individual (typically peripheral blood lymphocytes) are isolated and cultured in vitro. Cell division is then arrested using colcemid (which inhibits formation of the mitotic spindle); the cells are treated with hypotonic solution (to swell them and separate the chromosomes); a fixative is added (to kill and permeabilise the cells) and the chromosomes are stained with a chromatin-staining dye (e.g. Giemsa). Finally, the cells are ‘squashed’ on microscope slides and cells with clearly visible chromosomes (generally at metaphase in the cell cycle) are identified under the microscope. Homologous chromosomes have distinctive lengths, centromere positions and staining patterns, and these features are used to identify and organise the chromosomes into a karyotype (see Figure 2). By convention, the karyotype is constructed by pairing the autosomes and arranging them in order of size (from largest to smallest) and centromere position (metacentric→submetacentric→acrocentric→telocentric; see Figures 2 and 3). Image analysis software is often used to build and analyse the karyotype.
1. OBSERVING KARYOTYPES

Prepared slides

Examine slides of a metaphase preparation from white blood cells provided in the class. Locate a cell in which the chromosomes seem to be well spread out under the 40X objective, and then transfer to the 100X oil immersion lens. See if you can determine (a) what sex the donor was and (b) whether there is an abnormal number of chromosomes. (It may not be possible to determine this, but try).

Abnormalities in a karyotype

Any abnormalities in the individual’s karyotype (or idiogram) may be identified by comparing the karyotype with a normal reference karyotype. This enables the rapid detection of abnormal numbers of chromosomes (e.g. trisomy 21) and large changes in chromosome structure (e.g. deletions, inversions and translocations). However, small (<1Mbp) abnormalities are generally not visible and must be identified using molecular techniques.

Figure 2: Karyotype of a human male using Giemsa staining
Observing HeLa cell karyotypes

Your aim is to prepare and analyse metaphase chromosome spreads. You will be provided with a suspension of fixed cancer (HeLa) cells that have been commercially prepared for karyotyping. The HeLa cell line is widely used in cancer and cell biology research and has an interesting history: it was isolated without consent from an aggressive cervical carcinoma in the patient Henrietta Lacks in the 1950s, and became widely used in research as it is relatively easy to maintain in culture. (Why do you think this cell line is easy to culture?)

You will stain the prepared cells and mount them according to the following instructions. Work in pairs. (Note: This protocol is based on the documentation provided by CellServ for Kit 4 ‘Preparation of Human Chromosome Spreads’ available at www.cellservkits.com).

1. Place a microscope slide at a 45° angle on your bench.
2. Adjust a P1000 micropipette to 250 µl and attach a blue tip.
3. Gently resuspend the cells in the tube provided by slowly pipetting up and down.
4. Remove 250 µl of suspension from the tube. Hold the pipette 60 cm above the microscope slide. Allow one drop of cell suspension to “splat” onto the slide about 2 cm from the upper end and tumble down the slide. Carefully apply 6-8 more drops from various heights, one drop at a time, onto the same region of the slide.

It is important to release the cell suspension one drop at a time. Do not expel all of your cell suspension in one squirt, or you will obtain poor results. Gently blow across the slide (and away from yourself and others) for 2-3 seconds. This drying will help “spread” the chromosomes.
5. Allow the cells to AIR DRY **COMPLETELY**.

6. Dip the slide into the tube containing STAIN #1 for 1 second only. Repeat twice.

7. Drain off excess stain, wipe bottom of slide with paper towel (to minimise carryover) and dip the slide into STAIN #2 for 1 second only. Remove slide and repeat dipping twice.

8. Remove slide from stain and thoroughly rinse by dipping several times in 50ml tubes filled with distilled water.

9. Allow slide to AIR DRY **COMPLETELY**. Blowing may help speed up the drying process. Incomplete drying will result in very poor resolution.

10. Place a #1 coverslip over the slide and secure in place by painting around the edges of the coverslip with nail varnish. Allow to dry. You may wish to place coverslips side-by-side so as to allow viewing of the entire microscope slide.

11. Observe your slide using the 10X objective. Scan the spread for cells which appear to have ruptured and released their chromosomes and then shift to 40X objective to examine your spread more carefully. An ideal chromosome spread will contain distinct non-overlapping chromosomes with clearly visible sister chromatids (see Figure 3).

12. Count the number of chromosomes in 2-3 cells and answer these questions:

   - How many chromosomes would you expect to see in normal cells?

   - Do the HeLa cells contain a constant number of chromosomes? Explain your observations in terms of the differences between normal and cancer cells.

   - How could you reliably identify chromosomal abnormalities (if present) from your metaphase spreads?

![Figure 3: HeLa cell metaphase chromosome spread](image)
Suggested reading

Two books that give fascinating accounts of the history of HeLa cells:


2. **MITOSIS IN THE ONION ROOT TIP**

In today's lab you will be given a root tip of an onion plant. It was fixed (killed) by a mixture of acetic acid and alcohol and soaked for a short time in 70% ethanol to clear the cytoplasm of oil droplets and other material that might make the chromosomes difficult to see. It was then stained in aceto-carmine and stored in 45% acetic acid. This procedure destroyed the spindles and stained the chromosomes red.

**Preparing a root tip squash:**

This technique will be demonstrated in a video.

1. Place a root tip on a microscope slide and cover it with a drop of 45% acetic acid, the mounting medium.

2. If the root tip is thick, split it lengthwise. Keep one half on the present slide and transfer the other half to a drop of 45% acetic acid on a second slide. Thus, two slides can be made from one tip.

3. Hold the cut end of the root with a pair of forceps and cut off about 1 to 2 mm of the pointed tip, the deeply stained meristem, with a sharp razor blade. Discard the remainder of the root.

4. Cut the 1 to 2 mm of the tip remaining on your slide into 3 or 4 pieces. Spread these in the drop of acetic acid containing gum chloral to prevent drying out.

5. Cover the tip with a cover-slip. Avoid all movement of the cover slip from now on.

6. Hold the edge of the cover-slip with your fingers and tap the surface firmly with the blunt end of a pencil, dissecting needle or forceps to spread the cells - the red blobs of tissue should spread into pink smears.

7. Place the slide, cover-slip down, on a tissue then fold the tissue over the slide. Hold both ends of the slide firmly with one hand, and use the thumb of the other hand to press on the centre of the slide. It helps squash the cells if you roll your thumb slightly as long as you do not move the slide about.

8. Examine the whole preparation under the lowest power of the microscope and identify interesting cells.

*What makes a cell interesting? Can you see the chromosomes, and can you identify cells at different stages of the cell cycle?*
If the cells are not in a single layer, repeat the previous step. Switch to 40X objective and study the cell in detail. Return to low power when searching for other stages. This will speed your work immensely. Remember that you need to continually adjust the focus when using high power.

In the spaces below, draw a cell at metaphase (Figure 2) and a cell at anaphase (Figure 3). Write captions for your drawings and label them fully, including the following where appropriate: centromere, sister chromatids, daughter chromosomes. Sister chromatids are the two copies of a chromosome produced through DNA replication during S phase. They are attached to each other at the centromere until they separate during anaphase.

Figure 2:

Figure 3:
3. **MEIOSIS**

**Introduction:**

Sexual reproduction allows the genes of two individuals to combine and provides the variability upon which evolution can work. In sexually reproducing organisms, the production of sex cells, or gametes, requires that each parent's chromosomes be reduced to half the normal number.

This halving of the parent's chromosome number from the diploid, or 2n, number to the haploid, or n, number is the result of meiosis. Combining two haploid (n) gametes during fertilization then restores the chromosome number to the number that is characteristic of the diploid (2n) organism (Figure 4).

![Figure 4: Meiosis in animals](image)

Meiosis consists of two nuclear divisions (meiosis I and meiosis II) and results in the production of four daughter nuclei, each of which contains only half the number of chromosomes (and half the amount of DNA) characteristic of the parent (Figure 5).
Figure 5: Overview of meiosis. In this example, diploids have 2n=2 chromosomes.

During meiotic reduction of the chromosome the chromosomes are not just divided into two sets at random. In diploid organisms, chromosomes occur in matched pairs called homologous chromosomes. These are identical in size, shape, location of their centromeres, and types of genes present.

One member of each homologous pair is contributed by the male parent and one is contributed by the female parent during sexual reproduction. Meiosis provides a precise mechanism for separating these homologous chromosomes so that daughter cells always carry one member, or homologue, of each chromosome pair.
<table>
<thead>
<tr>
<th>How many cells form during the process of meiosis?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Are the cells formed in meiosis haploid (n) or diploid (2n)?</td>
</tr>
<tr>
<td>If the same set of chromosomes shown above were to undergo mitosis, would the resulting cells be haploid or diploid?</td>
</tr>
<tr>
<td>List three major differences between meiosis and mitosis:</td>
</tr>
<tr>
<td>1.</td>
</tr>
<tr>
<td>2.</td>
</tr>
<tr>
<td>3.</td>
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</table>
### Student Risk Assessment

<table>
<thead>
<tr>
<th>Hazards</th>
<th>Risks</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General</strong></td>
<td>See below</td>
<td><strong>At all times</strong> follow demonstrators instructions when handling all biological substances, chemicals and equipment items. Notify demonstrator immediately of any spills or other incidents.</td>
</tr>
<tr>
<td><strong>Sharps</strong></td>
<td>Physical injury</td>
<td>Use caution working with pipette tips. Dispose of used tips in the sharps bins provided on your bench.</td>
</tr>
<tr>
<td><strong>Use of electrical equipment</strong></td>
<td>Electrocution, fire, tripping over cables</td>
<td>Routine tagging and testing of equipment, visual inspection prior to use, do not use damaged equipment until repaired, don’t use near water or flammables.</td>
</tr>
<tr>
<td><strong>Chemicals</strong> (Taq DNA polymerase, Reaction buffer (10x), MgCl₂, dNTP’s, forward and reverse primers (oligonucleotides), template DNA (or cDNA), milliQ water)</td>
<td>Do not get in on skin or clothing. If you do, wash the affected area for several minutes with running water.</td>
<td>Wear Personal protective equipment (gowns, gloves and enclosed shoes). Wash hands before leaving the laboratory. <strong>Wear gloves at all times.</strong></td>
</tr>
</tbody>
</table>

### Emergency Procedures

In the event of an alarm sounding, stop the experiment, turn off electrical equipment and wait for confirmation from demonstrators to evacuate. Then pack up your bags and wash your hands. Follow the instructions of the demonstrators regarding exits and assembly points. The BioScience assembly point is in the lawn in front of the Chancellery. In the event of an injury inform the demonstrator. First aiders and contact details are on display by the lifts.

All used gloves should be placed in the biohazard bins. Used test tubes should be placed in the appropriate container on the front bench of the lab. **All solutions should be placed in the appropriate liquid waste containers.** Used pipette tips and microcentrifuge tubes should be placed in approved biohazard sharps containers provided on your bench.

I have read and understand the safety requirements for this practical class and I will observe these requirements.

Signature:…………………………………………………………………………Date:………………………………

Student number:……………………
OBJECTIVES

- Define and explain Mendel's law of segregation (the first law).
- Apply Mendel's first law to a simple cross between two heterozygous individuals.
- Demonstrate the alternative possible arrangements of homologous chromosomes during metaphase I of meiosis.
- Relate the arrangement of homologous chromosomes in metaphase I to the number of types of genetically different gametes that can be produced.
- Define & explain Mendel's law of independent assortment (the “2nd law”).
- Verify that the law of independent assortment holds true for alleles in a dihybrid cross between two heterozygous individuals.
- To successfully prepare samples for PCR.

MASTERING BIOLOGY TUTORIAL

Don’t forget your Mastering Biology quiz on DNA Structure and Replication, which you must complete by the end of next week. To prepare for this, work through the following BioFlix tutorial:

<table>
<thead>
<tr>
<th>ITEM TYPE</th>
<th>TITLE</th>
<th>MEDIAN TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tutorial</td>
<td>Meiosis (3 of 3): Determinants of Heredity and Genetic Variation (BioFlix tutorial)</td>
<td>9 min</td>
</tr>
</tbody>
</table>

CONTENTS

1. Inheritance of genetic information and Mendel's laws
2. Preparing for PCR
3. Investigating human pedigrees
1. INHERITANCE OF GENETIC INFORMATION AND MENDEL’S LAWS

Mendel’s first law: alleles segregate during meiosis (*Drosophila* genetics A)

Since there is a pair (the homologues) of each type of chromosome in a diploid organism, there will also be a pair of each type of gene: one gene on one chromosome and the second on its homologue. Genes for a particular trait are found at the same locus (physical place or location) on each of the homologous chromosomes. An allele is an alternative form of a gene. In some diploids, the two homologous copies of a gene are identical and such organisms are said to be homozygous. In contrast, in other organisms, the two copies are different; those organisms with different alleles at a locus are said to be heterozygous. An organism’s phenotype (its physical properties) depends in part on its genotype (the allelic state of its genes).

During meiosis, homologous chromosomes are separated from each other, and only one may be carried in a particular gamete or spore. Thus the gene copies carried on each of the homologous chromosomes are also separated or segregated.

When a diploid is heterozygous, this segregation is significant because the haploid gametes carry different alleles. Mendel’s first law states that alleles segregate in meiosis (Figure 1). When two haploid gametes combine during fertilization, two alleles for each trait are again present in the offspring.

Figure 1: Alleles segregate during meiosis.

*Why do the two chromatids of a chromosome have the same alleles (A and a) on one dyad?*
How are the A and a alleles distributed to the daughter cells?

Do your observations support Mendel's first law? Explain:

Using *Drosophila* to verify Mendel’s first law of segregation:

Mendel's first law can be verified by examining wing length in *Drosophila melanogaster* bred from the union of gametes from two parents that differ in a particular locus. In this example, there are two alleles, V and v. The phenotype of the VV homozygote is full size wings, the phenotype of the vv homozygote is vestigial or short wings, and the phenotype of the heterozygote (Vv) is full size wings. Because the VV and Vv genotypes have the same phenotype, the allele V is dominant, while v is recessive. By convention, the dominant allele is assigned the capital letter. We can also say the full size wing phenotype is dominant to the vestigial phenotype.

What size wings would the VV parent have?

What is the genotype of gametes produced by the VV parent?

What size wings would the vv parent have?

What is the genotype of gametes produced by the vv parent?

Consider the genotypes of gametes that can be produced by the homozygous parents, VV and vv. What would be the genotype of all offspring resulting from the union of one gamete from each parent?
What is the phenotype of the offspring in this generation (which is labeled F1)?

When F₁ individuals make gametes, their alleles for wing size will segregate. What are the genotypes of gametes produced by F₁ individuals?

The consequences of this segregation of alleles will become apparent when one examines the possible genotypes in the next generation (called F₂ since it is composed of the offspring produced by F₁ individuals).

The possible combinations of alleles that may be produced in each parent's gametes, and the results of these combinations in the genotypes of the offspring, can be determined by using a table called a Punnett square. All of the possible genotypes of gametes that can be produced by one parent are listed across the top of the square; all genotypes of gametes that can be produced by the other parent are listed along the side.

In the Punnett square below, one type of gamete from each F₁ parent has already been listed, and one possible combination is shown. Fill in the blanks for the other gamete genotype for each parent, and then complete the other three combinations in the square to determine the possible genotypes of the offspring. (Note: By convention, the dominant allele for each trait is written first: for example, Vv, not vV.) Below the Punnett square, list the genotypes and phenotypes of the four types of individuals produced in the F₂ generation.

<table>
<thead>
<tr>
<th>Gametes from father</th>
<th>Gametes from mother</th>
</tr>
</thead>
<tbody>
<tr>
<td>VV</td>
<td>V</td>
</tr>
<tr>
<td>Vv</td>
<td>VV (full)</td>
</tr>
<tr>
<td>Vv</td>
<td>Vv (full)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
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</tbody>
</table>
How many different kinds of genotypes are present in the F2 generation?

Indicate below the expected proportion of individuals with these genotypes:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Homozygous dominant</th>
<th>Heterozygous</th>
<th>Homozygous recessive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

How many different kinds of phenotypes are present in the F2 generation?

In the table below, fill in the expected proportion of individuals showing these phenotypes

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Proportion</th>
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<tbody>
<tr>
<td>Full wing</td>
<td></td>
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<tr>
<td>Short wing</td>
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</table>

Your demonstrator will supply you with a photograph simulation of randomly selected $F_2$ flies.

Record the total number of flies and then record number of flies with full size wings and those with short wings.

<table>
<thead>
<tr>
<th></th>
<th>Full wings</th>
<th>Short wings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Your data</td>
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<tr>
<td>Class total</td>
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How do these numbers compare to the proportion you predicted?
Mendel's second law: alleles of unlinked genes assort independently

Now let us consider meiosis involving two sets of homologous chromosomes. Alleles for trait A (A or a) are found on one pair of homologues. Alleles for an entirely different trait B (B or b) are found on the other pair of chromosomes. Assume that two parents are each heterozygous for both genes. Each parent would have the genotype AaBb. It is possible for these parents to produce gametes AB and ab or aB and Ab, depending on how the pairs of homologous chromosomes are arranged at metaphase I of meiosis.

The alleles for the two genes sort themselves out independently. The behaviour of A is not linked to that of B because the genes are on separate chromosomes (unlinked genes). So, for example, the combination AB is as likely as the combination ab. Mendel's second law states that alleles of unlinked genes assort independently (Figure 3).

Figure 3: Allele combinations in haploid gametes or spores are produced by independent assortment of alleles present on different chromosomes.

Since many gametes are produced at one time, a parent can produce gametes of all four genotypes: Ab, ab, aB, and Ab. When considering the possible genotypes for offspring, all gamete genotype possibilities for each parent must be considered.

Considering Mendel's second law, that alleles of unlinked genes assort independently:

*How many possible combinations of alleles exist if you consider the results from both possibilities above?*
How many different types of gametes can be made by an individual of genotype AaBb?

Mendel's second law can be verified by tracing the fate of two unlinked genes in *Drosophila* through a series of crosses. In addition to the locus for wing length (with alleles V and v) there is a locus that controls eye colour (with alleles S and s). Homozygous recessives (ss) have white eyes, while the other two genotypes have the dominant wild-type colour of red with a black glint in the centre. Suppose you cross a VVss mother with a vvSS father. This is called a dihybrid cross.

**Which alleles are present in the gametes of these parent types?**

Find the possible genotypes that would be present in individuals of the *F₁* generation by filling in the Punnett square below.

Find the possible genotypes that would be present in individuals of the *F₁* generation by filling in the Punnett square below.

<table>
<thead>
<tr>
<th>Gametes from father</th>
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<table>
<thead>
<tr>
<th>Gametes from mother</th>
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<th></th>
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</table>

**What is the genotype of all individuals in the F₁ generation?**

**What is the phenotype of all the individuals in the F₁ generation?**

**Which alleles are present in gametes produced by F₁ insects?**
Use the Punnett square below to find the proportions of different genotypes in the $F_2$ progeny resulting from all the possible unions of the various gametes produced by the $F_1$ generation.

Gametes from father

<p>| | | | |</p>
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Gametes from mother

<p>| | | | |</p>
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</tbody>
</table>

Circle all genotypes that result in a particular phenotype with the same wing size and eye colour.

Indicate the expected proportions (ratios) of individuals showing the following phenotypes:

- full wing, red eye
- full wing, white eye
- short wing, red eye
- short wing, white eye
Take another look at your flies (from the photograph simulation), particularly their eyes. Observe the number of flies with red eyes and those with white eyes. Now record the number of flies that have a) full wing + red eye, b) full wing + white eye, c) short wing + red eye, and d) short wing + white eye, and add your data to the class total.

<table>
<thead>
<tr>
<th></th>
<th>Full wing + white eye</th>
<th>Full wing + red eye</th>
<th>Short wing + white eye</th>
<th>Short wing + red eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Your data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class total</td>
<td></td>
<td></td>
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</tbody>
</table>

Do your results support what you predicted from the Punnett square? Explain:
3. **Setting up PCR**

The aim of this experiment is to introduce you to some molecular techniques that are used in medical diagnostics.

Perhaps the most important of these is PCR, which allows the amplification of specific gene sequences in any DNA sample, such as those collected for forensic and diagnostic screening. You will use this technique to screen some DNA samples for deletions in the Duchenne’s Muscular Dystrophy gene and determine a family pedigree for this disease. You will also precipitate DNA from an aqueous solution – probably the most commonly performed technique in a molecular lab.
Background:

In the last few years, PCR has become a very widely used technique in molecular biology. This technique enables the selective amplification of a desired DNA sequence. The process involves thermal cycling and DNA synthesis from oligonucleotide primers (see Figure 6).

In thermal cycling there are three different temperatures per cycle - a denaturation step which separates the DNA strands (usually 92 - 95°C); a step where the oligonucleotide primers anneal to the DNA template (generally 50 - 65°C); and a step at 72°C where the oligonucleotide primers are extended by Taq DNA polymerase. These three temperatures constitute one cycle and usually 25 - 35 cycles are used in each experiment.

The oligonucleotides provide the specificity for the reaction. They are usually between 20 and 30 bases in length, which is sufficiently long to hybridise (base pair) at only one sequence in the human genome. The oligonucleotides are synthesized chemically in an automatic machine.

Taq DNA polymerase is used because it is stable at high temperatures (92 - 95°C) and its temperature optimum is 72°C. It was originally isolated from a bacteria growing in thermal hot springs.

The PCR exponentially amplifies a DNA sequence. This is because in each cycle the number of DNA strands doubles and hence over a million-fold amplification can occur in 25 cycles. (See Figure 1 over page). In 1µg of human DNA (whose haploid genome contains 3 X 10^9 bps of DNA), a unique sequence of 300 bp comprises 0.1 pg of DNA which is too small a quantity to be seen on an agarose gel (of course it would be indistinguishable from the rest of the genome). If the 300 bp sequence can be selectively amplified a million-fold by PCR, then the 0.1 µg can be visualized on an agarose gel. This can be accomplished in an afternoon by the PCR technique.

DNA sequence:

The sequence that you will be attempting to amplify is an exon sequence from the Duchenne’s Muscular Dystrophy (DMD) gene. Within this particular family pedigree, there has been a deletion of approximately 200 bp within the coding sequence. Following PCR amplification of the specific DNA sequence, deletions of this size can be readily identified by agarose gel electrophoresis. Based on the experimental results you should be able to complete a pedigree for this family and determine the carriers and affected individuals and hence, the mode of transmission.
Figure 6: Outline of PCR.
Procedure:

Within a demonstrator group, you want to analyze the DNA from every member of the pedigree. Ensure that each student within your group has a different DNA sample to analyze so that all the DNA samples are analyzed (there are a total of 15 DNA samples to be analyzed in this pedigree).

1. Pipette 20µl of DNA into a 0.2 ml PCR tube.

2. Add 4 µl of PCR mix.

   This consists of (final concentration in 25 µl):
   - 2 pmole forward oligonucleotide primer
   - 2 pmole reverse oligonucleotide primer
   - 200 µM dATP, 200 µM dCTP, 200 µM dTTP, 200 µM dGTP
   - 16.6 mM (NH₄)₂SO₄
   - 67 mM Tris-HCl, pH 8.8 (at 25°C)
   - 6.7 mM MgCl₂

3. Add 1 µl of Taq DNA polymerase (supplied by your demonstrator).

4. Mix the contents of the tube by gently flicking the tube with your finger. Clearly label the tube with your initials and give the tube to your demonstrator.

5. The tube will be placed in the PCR machine for thermal cycling and will be returned to you at the next practical class.

6. Record here exactly what you did, including any mix-ups that might affect your results: you will not be penalised for these, but the information is necessary to interpret results properly next week.
3. INVESTIGATING HUMAN PEDIGREES

Introduction:

Once you have the data from your PCR experiment next week, you will use it to analyse a family pedigree. A study of human genetics is complicated by the fact that, unlike other species of animals or plants, our species is not bred experimentally and test crosses cannot be made to order. One of the principal tools is the pedigree, a phenotypic record of a family extending over several generations, showing whether each individual is affected by some condition. We can use a standard format for such a pedigree so that everyone can understand it. A standard set of symbols is used in the pedigree shown in Figure 7.

Each individual is identified by the generation, and the relative order of appearance within that generation. Hence III 2 is the last individual shown in this pedigree. Affected means that the individuals show some unusual condition, and symbols for these individuals are shaded in the pedigree. Shading over only half of a symbol indicates individuals who are known heterozygotes (carriers).
Analysing for a single-locus disorder such as Duchenne’s Muscular Dystrophy:

Single locus disorders can be inherited in a number of ways, depending on whether they are dominant or recessive, or whether they are X-linked. Table 1 below shows a list of possible modes of inheritance, and their outcomes. This assumes that the abnormal alleles are very rare in the general population, which is true for most single locus disorders.

*What mode of inheritance is most likely for the disorder shown in Figure 7?*

Table 1.

<table>
<thead>
<tr>
<th>Mode of Inheritance</th>
<th>Transmission</th>
<th>Parents of Affected</th>
<th>Siblings of Affected</th>
<th>Offspring of Affected</th>
<th>Population Sex Ratio of Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autosomal Dominant</td>
<td>Never skips a generation</td>
<td>Aa x aa</td>
<td>1/2 affected</td>
<td>1/2 affected</td>
<td>1:1</td>
</tr>
<tr>
<td>X-linked Dominant</td>
<td>Never skips a generation</td>
<td>Affected father B- x bb or Affected mother b- x Bb</td>
<td>All sisters affected, all brothers normal or 1/2 affected (both sexes)</td>
<td>Depends on sex of affected, as for previous generation</td>
<td>M:F</td>
</tr>
<tr>
<td>X-linked Recessive</td>
<td>Skips generations (through female carriers)</td>
<td>D- x Dd</td>
<td>All sisters normal, 1/2 brothers affected</td>
<td>All normal</td>
<td>M:F</td>
</tr>
<tr>
<td>Autosomal Recessive</td>
<td>Skips generation</td>
<td>Ee x Ee may be related</td>
<td>1/4 affected (both sexes)</td>
<td>All normal</td>
<td>1:1</td>
</tr>
<tr>
<td>Y-linked (very unlikely)</td>
<td>Male to male</td>
<td>F- x --</td>
<td>All brothers affected</td>
<td>All sons affected</td>
<td>M only</td>
</tr>
</tbody>
</table>
Investigation of colour blindness:

Your demonstrators will test you for colour blindness, a fairly common genetic variant. Record the information below:

Table 2:

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total number tested</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Number of colour blind</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Incidence of colour blindness (% total tested)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Proportion of total colour blind individuals</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Can you speculate as to the mode of transmission of colour blindness? Suggest how you might confirm this.*

Investigation of common Mendelian variants in humans:

Many of us have common genetic variations that are harmless but help to make us individual. Look at Table 11.4.2, and inspect as many members of the class as you can for each trait. Record the numbers of each variant you find, together with the gender of your subjects, and the frequency with which you find more than one variant in any one person. Can you deduce any rules for inheritance of any of these?
Table 3.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Widow’s peak</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a V-shaped hairline above the forehead)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cleft chin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a Y-shaped furrow on the chin)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mid-digital hair</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(hair on the middle joints of the fingers: may be very fine)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ear lobes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(the lobes of the ears can be free or attached: record those attached)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tongue rolling</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(the ability to roll one’s tongue into a tube)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Darwin’s tubercle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a small lump on the rim of the external ear)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Can you make any suggestions as to the mode of inheritance of any of these traits? Explain:
### Practical 9: Genes 3 – Genetic Screening by PCR 2

#### Student Risk Assessment

<table>
<thead>
<tr>
<th>Hazard</th>
<th>Risks</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrical hazard (agarose gel electrophoresis)</td>
<td>Electric shock</td>
<td>Electrophoresis equipment has been tested, tagged and certified for use in the Laboratory. “Danger High Voltage” warning signs and safety interlocks are fitted on electrophoresis tanks.</td>
</tr>
<tr>
<td>Hazardous chemicals (Gel Red)</td>
<td>Ingestion May be harmful if swallowed.</td>
<td>Personal Protection: Wear appropriate safety goggles, gloves and protective clothing. In case of skin contact Wash off with soap and plenty of water. Consult a physician. In case of eye contact Flush eyes with water as a precaution. If swallowed Do NOT induce vomiting. Rinse mouth with water.</td>
</tr>
<tr>
<td>UV light (Gel doc transilluminator)</td>
<td>UV damage to skin and/or eyes</td>
<td>All unshielded transilluminators have been removed from undergraduate teaching labs. Ensure UV switches off when door is opened – if not, switch off equipment and immediately notify staff.</td>
</tr>
</tbody>
</table>

### Personal Protective Equipment

- Closed in Footwear
- Lab. Coat
- Gloves
- Safety glasses

### Emergency Procedures

In the event of an alarm sounding, stop the experiment, turn of electrical equipment and wait for confirmation from demonstrators to evacuate. Then pack up your bags and wash your hands. Follow the instructions of the demonstrators regarding exits and assembly points. The BioScience assembly point is in the lawn in front of the Chancellery. In the event of an injury inform the demonstrator. First aiders and contact details are on display by the lifts.

All used microfuge tubes should be placed in the biohazard bins. Used test tubes should be placed in the appropriate container on the front bench of the lab. All solutions should be placed in the appropriate liquid waste containers. Used slides and cover slips should be placed in approved biohazard sharps containers.

I have read and understand the safety requirements for this practical class and I will observe these requirements.

Signature: ................................................................. Date: .............................................
Student number: .............................................
OBJECTIVES

Same as for last week.

CONTENTS

1. Completion and analysis of screening PCR
2. Precipitation of genomic DNA from peas
3. Post-lab discussion

1. COMPLETION AND ANALYSIS OF SCREENING PCR

Identification of hazards:

There are some potential low-level risks associated with this practical class as follows:

Chemical Hazards                  GelRed™ – fluorescent nucleic acid-binding dye
Biological Hazards                Human DNA
Procedural Hazards                UV irradiation (photographing agarose gel)
                                   High voltage electric current (electrophoresis power packs)

Overview:

Following amplification of an exon from the DMD gene by PCR last week, you now need to visualise the amplified DNA fragments. This is accomplished by separating the DNA fragments using agarose gel electrophoresis and staining the samples to visualise the DNA. You will prepare the gels, load your DNA, run the gels and visualise the DNA.

Background

Due to its repetitive structure, native double-stranded DNA has a constant charge per unit length and, on average, a constant mass per unit length. DNA molecules, because of their identical shape, will migrate in an electric field at a rate inversely proportional to their length or mass. Consequently, one of the simplest and most rapid means of separating DNA fragments of varying sizes is by electrophoresis in an agarose gel using an alkaline buffer.
The DNA fragments are highly negatively charged and so migrate to the positive electrode. Agarose is a complex mixture of polysaccharides isolated from seaweed. When the agarose is heated in solution it will form a gel as it cools (like jelly). The agarose provides a matrix where the pore size can be varied depending on the percentage agarose in the gel. For example, a 0.7% gel will separate kilobase sized fragments whereas a 1.5% gel can be used for fragments 100 –1000 base pairs (these are very general estimates). The gels are usually produced as a horizontal slab (approx. 4mm thick) with GelRed™ used to detect the DNA. GelRed™ is a dye molecule that binds to nucleic acids and produces a luminescence under ultraviolet light. Very small amounts of DNA (less than 100ng) can be detected by this method. It is possible to estimate the size of DNA fragments by observing the distance of migration relative to the migration of standard DNA molecules of known size.

**Procedure:**

Agarose gels will be prepared for you before the class.

You should perform the following steps. Each bench group should have two gels.

1. If not already done so, place the gel in the electrophoresis tank. Make sure that the top of the gel (the end with the comb) is next to the negative electrode (black) i.e. NOT at the end with the positive electrode.

2. Add enough running buffer to just cover the gel.

3. Carefully remove the comb from the gel and ensure that buffer enters the wells that have been formed when the comb was removed. There should be no air bubbles in the wells.

4. Add 5 µl of gel loading buffer (GLB) to your PCR sample.

5. Set the pipette to 6 µl and gently pipette the DNA/GLB mixture up and down to ensure they are completely mixed.

6. Pipette 6 µl of this mix into the wells in the gel. Make sure you make a record of which sample is loaded in which lane.

7. In the middle lane of the gel, load the molecular weight markers (labelled M).

8. When all the samples are loaded, place the lid on the electrophoresis apparatus and attach the electrodes to the power supply using the leads provided. Make sure that the top of the gel is attached to the negative electrode (black) so that the negatively charged DNA will migrate through the gel to the positive electrode.

9. Run the gel (at 100 volts) until the bromophenol blue marker dye has migrated half way.

10. While this is happening, perform the DNA precipitation (part 2 of this practical).
11. With the aid of your demonstrator, visualise the DNA in the gel using the Gel Documentation system.

**Analysis:**

Estimate the size of the bands on the agarose gel, using the molecular weight markers as a guide. The sizes of the marker bands are: 1000, 800, 600, 500, 400, 300, 200, 150, and 100 base pairs, with an additional faint band at 50 base pairs. With this information, you should be able to determine which individuals in the pedigree have a complete exon and which have a deletion. Use this to complete the following pedigree.

```
I
  1
  2
II
  1
  2
  3
  4
III
  1
  2
  3
  4
  5
IV
  1
  2
  3
  4
```

What is the mode of transmission of Duchenne's Muscular Dystrophy?
2. PRECIPITATION OF GENOMIC DNA FROM PEAS

Although DNA is packed so efficiently into cells that we cannot see it, it is possible to isolate DNA from cells and precipitate the DNA from solution so that it is visible. Indeed DNA can be isolated from almost any organism, including the food we eat (provided it hasn’t been cooked!).

DNA has been prepared for you from peas by using high salt solution and detergent to lyse the cells, extract the nuclei, and release the DNA into solution.

You will precipitate the DNA from this aqueous solution by the addition of cold alcohol.

1. Pipette approximately 1 ml of the aqueous DNA solution into a clean specimen tube.

2. Using the 1 ml disposable plastic pipette, slowly add 2 ml of cold 95% ethanol. Let the ethanol run down the side of the tube so that it forms a layer above the aqueous DNA solution.

3. Using the pipette gently stir the layer where the ethanol touches the DNA solution. You should observe the formation of long fibrous strands of DNA.

4. If you are careful, you should be able to pull the DNA out of the test tube by gently swirling the pipette in the DNA layer and then pulling it through the alcohol layer.

**What colour is your DNA?**

Pure DNA should be translucent. If it is whitish in colour then it still has some proteins (called histones) attached to it.

**Why does DNA appear stringy?**

If you want to keep the DNA, gently ease it off the end of the pipette into a vial of 50% ethanol. Cap the vial tightly. In the PCR you used GelRed™ to visualise your DNA under UV light.

**What do you see? Are they the same or different?**
3. LAB DISCUSSION: GENETICS

Introduction

Today's activity will be conducted according to the “think, pair, square” model. Initially each student should individually consider the question for about 5 minutes. The students should then pair up and further explore the question for another 5-10 minutes. Then finally the whole group should come together.

Topic (other questions may be added)

What investigations should be undertaken if we are to decide whether or not male and female sporting competition should be restricted to people who are XY and XX respectively?
WEEK 13

Biological modelling

In order to complete this task, you will be assigned to a small group of four students within your demonstrator group. Together, you need to choose a major biological system or concept that you will explain in class by presenting and demonstrating a model. You will need to meet both in and outside class time to design and construct your model. The attendance, roles, discussion, allocation of duties etc. of these meetings must be documented by completing a downloadable template from Moodle. Your notes may also need to document communications made via email / online discussions. Details on page 35.

PLEASE NOTE that if you miss this assessment due to illness or misadventure, you must apply for special consideration online (see page 19 for instructions).