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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 three interconnected learning components: a lecture series, a laboratory program, and online activities. 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 that revise and reinforce the concepts covered in the course.

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 and safety glasses (available at various stores on campus) and complete an online Health and Safety quiz. You must come to your timetabled lab class with a copy of the Course Manual, appropriate stationery and wearing closed shoes. 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, let your demonstrator know and we can arrange one for you.

To ensure you are organised for the year, look ahead in this outline for the due dates of assignments and enter them into your diary. Details of the assignments are included in this Course Manual and further information may 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 us at BABS1201@unsw.edu.au. Our laboratory classes are run relatively informally, so you are welcome to ask questions and discuss the material with your convenors, demonstrators and the technical staff in class as needed.

We hope your study of biology this term 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 forums 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 as clearly and respectfully as you would like us to answer it!

• 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 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. Use the Lightboard revision videos available via Moodle, if applicable.
  4. Post your question on a 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 as they are only employed for their face-to-face hours. You can instead post your question to the Moodle forums for your convenor or the course administrator to answer. 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 provided throughout the course and the Lightboard videos and quizzes 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-term exam. What should I do?
If you miss the mid-term 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 15 for instructions). You will be notified of the outcome of your application before the end of term 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 15 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 term 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 15 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 15 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 15 for details of the supplementary exam.
# BABS1201 Weekly Class and Assessment Schedule – T1, 2019

<table>
<thead>
<tr>
<th>Week No.</th>
<th>Week Commencing</th>
<th><strong>LECTURE A</strong></th>
<th><strong>LECTURE B</strong></th>
<th><strong>LECTURE C</strong></th>
<th>Laboratory</th>
<th>Assessments &amp; online activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18-Feb</td>
<td>1. Introducing BABS1201 RLB &amp; JW</td>
<td>2. Life - RLB</td>
<td>3. Cells I - RLB</td>
<td>Laboratory introduction</td>
<td>Health and safety quiz</td>
</tr>
<tr>
<td>6</td>
<td>25-Mar</td>
<td>REVISION - RLB</td>
<td>15. DNA Replication - LLM</td>
<td>16. Cell division and reproduction - LLM</td>
<td>MID-TERM TEST</td>
<td>Mid-term test (15%)</td>
</tr>
<tr>
<td>7</td>
<td>1-Apr</td>
<td>17. Gene expression I - LLM</td>
<td>18. Gene expression II - LLM</td>
<td>19. Polymerase Chain Reaction - LLM</td>
<td>Prac 5 Mitosis &amp; cell division</td>
<td>Pre-lab 5 quiz Quiz 3 (1%) Pitch in lab (5%)</td>
</tr>
<tr>
<td>10</td>
<td>22-Apr</td>
<td>PUBLIC HOLIDAY</td>
<td>PUBLIC HOLIDAY</td>
<td>PUBLIC HOLIDAY</td>
<td>Presentations (Friday students)</td>
<td>Presentations (15%) and portfolios (5%) in lab</td>
</tr>
<tr>
<td>11</td>
<td>29-Apr</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Quiz 5 (1%)</td>
</tr>
</tbody>
</table>

JW: John Wilson, RLB: Rebecca LeBard, LLM: Louise Lutze-Mann, AMG: Anne Galea, VS: Vladimir Sytnyk, PW: Paul Waters
<table>
<thead>
<tr>
<th>Course Identity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Course Code</strong></td>
<td>BABS1201</td>
</tr>
<tr>
<td><strong>Course Name</strong></td>
<td>Molecules, Cells &amp; Genes</td>
</tr>
<tr>
<td><strong>Academic Unit</strong></td>
<td>School of Biotechnology and Biomolecular Sciences</td>
</tr>
<tr>
<td><strong>Level of Course</strong></td>
<td>First Year Undergraduate</td>
</tr>
<tr>
<td><strong>Units of Credit</strong></td>
<td>6</td>
</tr>
<tr>
<td><strong>Assumed Knowledge, Prerequisites or Co-requisites</strong></td>
<td>Nil</td>
</tr>
<tr>
<td><strong>Hours per Week</strong></td>
<td>6</td>
</tr>
<tr>
<td><strong>Number of Weeks</strong></td>
<td>10 weeks</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Course Convenors</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr Rebecca LeBard</td>
<td>Room 103, Level 1, Biological Sciences Building (eastern end)</td>
</tr>
<tr>
<td></td>
<td>(02) 9385 2026; Meetings by appointment only. Email: <a href="mailto:r.lebard@unsw.edu.au">r.lebard@unsw.edu.au</a></td>
</tr>
<tr>
<td>John Wilson</td>
<td>Room G27 (inside the BSB Office), Biological Sciences Building</td>
</tr>
<tr>
<td></td>
<td>(02) 9385 8156; Meetings by appointment only. Email: <a href="mailto:j.e.wilson@unsw.edu.au">j.e.wilson@unsw.edu.au</a></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Administrative Support (enrolment queries, academic advise etc)</th>
<th>E-mail: <a href="mailto:BABS1201@unsw.edu.au">BABS1201@unsw.edu.au</a></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>School student office</td>
</tr>
<tr>
<td></td>
<td>Room G27, Ground floor, Biological Sciences Building</td>
</tr>
<tr>
<td></td>
<td>(02) 9385 8047; Opening Hours: 9:00am – 4:30pm, Mon-Fri. Questions: UNSW.to/webforms</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Course Resources</th>
<th></th>
</tr>
</thead>
</table>

Multiple hard copies are available from the UNSW library and they have an electronic version. Search the library catalogue for "Campbell Biology Australian and New Zealand Edition." |
| **Course Web Site** | BABS1201 uses Moodle as the learning management system. This contains background information, links to resources, lecture notes, and discussion forums. Once you are enrolled in BABS1201, you can access the Moodle site at: [https://moodle.telt.unsw.edu.au/login/index.php](https://moodle.telt.unsw.edu.au/login/index.php)  
Your username is your student number preceded by a lower-case ‘z’ e.g. z1234567.  
Your password is your zpass. |
| **Course Manual** | A course manual is required and may be purchased from the UNSW Bookshop or downloaded from the BABS1201 Moodle site. |
| **Practical Class Requirements** | For all practical classes students are required to:  
• Bring with them the complete BABS1201 Molecules, Cells & Genes Course Manual.  
• Read the relevant practical before class and complete the pre-lab quiz.  
• Bring 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 improperly 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.  
• Arrive on time. You will not be permitted to enter once the class has commenced. |
# 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>A. Online Laboratory Safety Quiz</td>
<td>This online laboratory safety quiz is accessible through the BABS1201 Moodle site. It is COMPULSORY to complete this quiz with a mark of 100%.</td>
<td>Week 1</td>
<td>N/A</td>
</tr>
<tr>
<td>B. Online quizzes</td>
<td>Online quizzes are designed to provide you with formative feedback on how you are progressing in the course. Each task is a short quiz worth 1% of your final assessment for the course.</td>
<td>Weeks 3, 5, 7, 9 and 11</td>
<td>5%</td>
</tr>
<tr>
<td>C. Science Communication Project</td>
<td><strong>ESSAY.</strong> The essay should be approximately 1000 words long, excluding references. You must prepare and submit your INDIVIDUAL essay online via the Turnitin plagiarism checking software in Moodle. Check Moodle announcements regularly for any changes.</td>
<td>Practical 4 Week 5</td>
<td>15%</td>
</tr>
<tr>
<td></td>
<td><strong>PITCH.</strong> TEAM presentation of ideas to your laboratory group for peer feedback on the design, biological content and feasibility of their project whilst providing similar feedback to other project teams in the class.</td>
<td>Practical 5 Week 7</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td><strong>PRESENTATION.</strong> Utilise the peer feedback received in the pitch to finalise your TEAM presentation on your biology topic for submission and showcasing at the end of term.</td>
<td>Week 9*</td>
<td>15%</td>
</tr>
<tr>
<td></td>
<td><strong>PORTFOLIO.</strong> TEAM submission documenting the proceedings of all team meetings, including an inventory of ideas, team member roles, major decisions, and other notes on the presentation design and execution.</td>
<td>Week 9*</td>
<td>5%</td>
</tr>
<tr>
<td>D. Mid-term test</td>
<td><strong>Duration:</strong> 45 minutes. <strong>Format:</strong> Multiple choice questions. <strong>Content:</strong> All theory and practical material from Weeks 1-5 (inclusive). <strong>Venue:</strong> your enrolled lab <strong>Time:</strong> your enrolled lab</td>
<td>Week 6</td>
<td>15%</td>
</tr>
<tr>
<td>E. Final Theory Exam</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 1-10.</td>
<td>Final exam period (Date to be advised by exams branch)</td>
<td>40%</td>
</tr>
</tbody>
</table>

Further details on the assessments are provided in the “Assessments” section of Moodle and on page 17.

* Students with a laboratory class on Friday present and hand in their portfolios in Week 10 due to the Good Friday holiday.

## Course Themes

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 term, 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:

- Introduction to laboratory safety
- Exploring cell structure (Practicals 1-2)
- Exploring cell function (Practicals 3-4)
- Exploring genes (Practicals 5-6)

There are aims 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 attendance at EVERY laboratory class is COMPULSORY, including the introductory term 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 3 and Expectations of Students on page 17 for details on absences from classes.

Lectures

A uniform set of lecture notes is provided for each lecture, that provides the learning outcomes and relevant textbook references. This is provided particularly for those students that wish to prepare ahead of time and the notes cover all assessable material. Please note that your lecturers will often provide another set that they use in class, these may extend the information for those interested and provide more context and examples from research. At UNSW, the people who teach you biology have made significant contributions to your area of study.

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 in person.

If you choose to attend, please be quiet when the lecturer is speaking. Chatting makes it difficult for your fellow students 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 academic misconduct. 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 and recordings are accessible via the BABS1201 Moodle site.

UNSW Science Graduate Attributes Developed in this Course
<table>
<thead>
<tr>
<th>Science Graduate Attributes</th>
<th>0 = no focus</th>
<th>1 = minimal</th>
<th>2 = minor</th>
<th>3 = major</th>
<th>Activities / Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Research, inquiry and analytical thinking abilities</td>
<td>3</td>
<td>Guided laboratory practicals; independent and collaborative lab research; and independent research.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capability and motivation for intellectual development</td>
<td>3</td>
<td>Small group discussions; essay and project presentation.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethical, social and professional understanding</td>
<td>2</td>
<td>Science communication topics may address some of the ethical and social issues of biology, and the “how to think like a scientist” theme throughout the course addresses professional understanding.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Communication</td>
<td>3</td>
<td>Development of scientific writing skills through introduction to scientific literature (essay); and the project.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Teamwork, collaborative and management skills</td>
<td>3</td>
<td>Team independent research project; facilitation of group discussions in class and on Moodle.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Information literacy</td>
<td>3</td>
<td>Introduction to finding reviews and primary scientific literature (essay), team project.</td>
<td></td>
<td></td>
<td></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 practise 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.

**Lecture Program**

Please note that any changes to learning outcomes will be communicated via Moodle.
<table>
<thead>
<tr>
<th>Introducing BABS1201</th>
<th>In this lecture you will meet your coordinators, receive information on the course structure, expectations, assessments and procedures.</th>
</tr>
</thead>
</table>
| **Scientific Literature** | LO1 Explain the purpose of a scientific journal  
LO2 Identify a primary article and list its features  
LO3 Explain the differences between primary and secondary sources of scientific literature  
LO4 Describe the term ‘peer review’ as it applies to scientific literature |
| **Scientific Communication** | Introduces the Biology Threshold Learning outcomes, see pg 17-18. |
| **Life** | LO1 List the major elements of life.  
LO2 Describe some properties of water that make it essential for life as we know it.  
LO3 Changes in pH affect living organisms |
| **Cells I** | LO1 List the major elements of life  
LO2 Explain how the diversity of life is classified  
LO3 Define what a cell is  
LO4 List some characteristics of life  
LO5 Explain the fundamental differences between prokaryotes and eukaryotes  
LO6 Describe the concept of endosymbiosis |
| **Cells II** | LO1 Identify characteristic structures of eukaryotic and bacterial cells, and describe their basic functions  
LO2 Describe the endomembrane system  
LO3 List the main components of the cytoskeleton and briefly describe their roles in the cell |
| **Macromolecules I (CHO & lipids), II (proteins) and III (nucleic acids)** | LO1 Provide a broad definition of the term “macromolecule”.  
LO2 Explain the way in which macromolecules are generally synthesised and broken down by organisms.  
LO3 Briefly describe the general structural features of each of the four major classes of macromolecules.  
LO4 List some examples of members belonging to each of the four major classes of macromolecules.  
LO5 Describe some of the key functions of members belonging to each of the four major classes of macromolecules. |
| **Cell Integrity** | LO1 To describe the structure of cell membranes and their function in cell integrity.  
LO2 To describe the different components of the cell membrane that are important in maintaining cell integrity.  
LO3 To explain the non-selective diffusion of some small molecules across cell membranes and osmosis. |
| **Cellular transport** | LO1 To explain the mechanisms by which small molecules may be selectively transported in and out of cells.  
LO2 To describe the concept of a membrane potential arising from ionic imbalances across cell membranes.  
LO3 To describe the different types of endocytosis. |
| **Metabolism I: Metabolic concepts** | LO1 Explain the terms metabolism, catabolism and anabolism.  
LO2 Explain the basic model for enzyme catalysis  
LO3 Describe the structure and function of ATP  
LO4 Describe the terms respiration and fermentation.  
LO5 Describe the importance of redox reactions in metabolism, including the common cofactors. |
<p>| <strong>Metabolism II:</strong> | LO1 Describe the catabolism of different macromolecules. |</p>
<table>
<thead>
<tr>
<th>Topic</th>
<th>LO1</th>
<th>LO2</th>
<th>LO3</th>
<th>LO4</th>
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<tr>
<td><strong>Extracting energy from food</strong></td>
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<td><strong>Photosynthesis: Synthesising food from energy</strong></td>
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<td><strong>DNA replication</strong></td>
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<td><strong>Gene expression I: Transcription</strong></td>
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<td><strong>Gene expression II: Translation</strong></td>
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<td><strong>The polymerase chain reaction</strong></td>
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<td><strong>Mendel's laws of heredity</strong></td>
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<td><strong>Mechanisms of inheritance</strong></td>
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<td><strong>Population genetics</strong></td>
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**Extracting energy from food**

LO2 Describe the central features of glycolysis, the TCA cycle and oxidative phosphorylation.
LO3 Explain the process of chemiosmosis.
LO4 Compare the advantages and disadvantages of fermentation and aerobic respiration.
LO5 Explain the control of cellular respiration via feedback.

**Photosynthesis: Synthesising food from energy**

LO1 To comprehend the functions of the different stages in photosynthesis light harvesting, the conversion of light energy into chemical energy and carbon dioxide fixation.
LO2 To comprehend the overall organisation of the light reactions in photosystems I and II.
LO3 To describe, in overview, the fixation of carbon dioxide and synthesis of glucose in the Calvin cycle.
LO4 To compare and contrast the generation of energy from photosynthesis and oxidative phosphorylation.

**DNA replication**

LO1 Explain the semi-conservative model of DNA replication.
LO2 Describe the basic steps involved in the process of DNA replication.
LO3 Describe the function of the major enzymes involved in DNA replication.

**Cell division and reproduction**

LO1 To explain the difference between a gene and a chromosome and describe the way in which DNA is packaged within a cell.
LO2 To explain the processes of mitosis and meiosis and the differences between them.

**Gene expression I: Transcription**

LO1 Describe the genetic code.
LO2 Explain how the instructions contained within DNA are transcribed into RNA.
LO3 Define the three stages of transcription.
LO4 State the main differences in gene expression between bacteria and eukaryotes.

**Gene expression II: Translation**

LO1 Explain the process of translation, and relate it to cell function and the role of ribosomes.
LO2 Describe the basic structure and function of tRNA.
LO3 Describe the three main stages of translation.
LO4 Explain the main differences between control of gene expression in bacteria and eukaryotes.

**The polymerase chain reaction**

LO1. To describe the PCR including the steps involved. LO2. To list several applications of PCR.

**Mutation**

LO1 To explain at mechanisms by which mutations can arise.
LO2 To elate the occurrence of mutations to the outcomes for cells and whole organisms.
LO3 To appreciate the importance of the rate of mutation for the evolution of species.

**Mendel's laws of heredity**

LO1 To describe Mendel's laws.
LO2 To explain the basis of inherited characteristics.
LO3 To explain why genotype does not always equal phenotype.

**Mechanisms of inheritance**

LO1 To explain the varied modes of inheritance in difference organisms. This includes the concepts of dominant and recessive traits, sex-linked and autosomal inheritance, linkage and recombination, complex traits eg codominance

**Population genetics**

LO1 To explain the evolutionary forces that influence population genetics using the Hardy-Weinberg model.
# 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) and actively participated including by keeping an up-to-date laboratory manual recording all data and completion of calculations and questions.

Holidays 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

<table>
<thead>
<tr>
<th>BABS School Contact</th>
<th>Marc Wilkins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><a href="mailto:m.wilkins@unsw.edu.au">m.wilkins@unsw.edu.au</a></td>
</tr>
<tr>
<td></td>
<td>Tel: 9385 3633</td>
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<table>
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<tr>
<th>Science Faculty Contact</th>
<th>Gavin Edwards</th>
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</thead>
<tbody>
<tr>
<td>Associate Dean (Undergraduate programs)</td>
<td><a href="mailto:g.edwards@unsw.edu.au">g.edwards@unsw.edu.au</a></td>
</tr>
<tr>
<td>Tel: 9385 7111</td>
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<table>
<thead>
<tr>
<th>University Contact</th>
<th>Student Conduct and Appeals Officer (SCAO)</th>
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<tbody>
<tr>
<td></td>
<td>within the Office of the Pro-Vice-Chancellor (Students)</td>
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<td></td>
<td>Registrar Tel: 9385 8515</td>
</tr>
<tr>
<td></td>
<td><a href="mailto:studentcomplaints@unsw.edu.au">studentcomplaints@unsw.edu.au</a></td>
</tr>
</tbody>
</table>

## Special Consideration and Further Assessment

### Explanation

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

You must submit the application prior to the start of the relevant exam, or before a piece of assessment is due, except where illness or misadventure prevent you from doing so. If you become unwell on the day of the exam or fall sick during an exam, you must provide evidence dated within 24 hours of the exam, with your application.

UNSW has a fit to sit/submit rule which means that if you sit an exam or submit a piece of assessment, you are declaring yourself fit to do so.

You must obtain and attach Third Party documentation before submitting the application. Failure to do so may result in the application being rejected.

The application must be made through Online Services in myUNSW (My Student Profile tab > My Student Services > Online Services > Special Consideration).

### 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.**

The BABS1201 Supplementary Examination Period will be:

**Monday 27 May – Friday 31 May, 2019**

Further assessment exams will be offered on ONE day in this period **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, web site, 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.

### Course Assessment – Detailed Instructions

#### A. ONLINE LABORATORY SAFETY QUIZ

In order to be permitted to take part in laboratory classes, you must also complete an online Laboratory Safety Quiz that is accessed through the BABS1201 Moodle site.

When you have finished the quiz and submitted all your answers, you will receive a mark out of 10. 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 10/10. 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.
If you have not scored 100% in the quiz you will NOT be permitted to attend lab classes until you have satisfied this requirement.

B. ONLINE QUIZZES

Online quizzes are designed to provide you with formative feedback on how you are progressing in the course. Each task is a short quiz worth 1% of your final assessment for the course.

Each quiz is open for two weeks, with the due dates on Friday of weeks 3, 5, 7, 9 and 11. You are strongly encouraged to complete the quiz ahead of the deadline.

C. SCIENCE COMMUNICATION PROJECT

The Science Communication Project is a group assignment with multiple components that runs throughout the term. The project focuses on advanced biology topics and aims to develop, exercise and enhance your science communication skills. Working in a team of four (4), students you will:

1. Select a topic from the list provided;
2. Perform a literature search on the chosen topic (see Moodle for a module on how to conduct a literature search);
3. Write and submit an individual ESSAY (15%) that compares primary and secondary scientific journal articles on their topic (see Moodle for a module on scientific literature that describes primary and secondary scientific journal articles);
4. Meet regularly with your team to discuss findings on your topic, and develop a presentation that effectively communicates the central biological concepts, especially with respect to how those concepts align with BABS1201 course themes and learning activity topics;
5. PITCH (5%) your presentation ideas to the larger laboratory group for peer feedback on the design, biological content and feasibility of their project whilst providing similar feedback to other project teams in the class;
6. Utilise the peer feedback received in the pitch to finalise your 10 minute team PRESENTATION (15%) on your biology topic for submission and showcasing at the end of term;
7. Submit a team PORTFOLIO (5%) that documents the proceedings of all team meetings, including an inventory of ideas, team member roles, major decisions, and other notes on the presentation design and execution;

This project incorporates the following UNSW Science graduate attributes:

- Research, inquiry and analytical thinking abilities;
- Capability and motivation for intellectual development;
- Communication;
- Teamwork, collaborative and management skills; and,
- Information literacy.

This project also incorporates many of the Biology Threshold Learning Outcomes recognised by the Australian Council of Deans of Science:

Upon completion of a bachelor degree or major in biology, graduates will:
### Understanding biology
1.1 Demonstrate a coherent understanding of biology by articulating the methods of biology, and explaining why current biological knowledge is both contestable and testable through further inquiry.
1.2 Demonstrate a coherent understanding of biology by explaining the role and relevance of biology in society.
1.3 Recognise that biological knowledge has been acquired by curiosity and creativity, and demonstrate creativity in thinking and problem solving.
1.4 Recognise and appreciate the significant role of biodiversity in sustaining life on our planet.

### Biological knowledge
2.1 Exhibit depth and breadth of biological knowledge by demonstrating well-developed understanding of identified core concepts in biology.
2.2 Exhibit depth and breadth of biological knowledge by demonstrating that these ‘core concepts’ have interdisciplinary connections with other disciplines.

### Inquiry and problem solving
3.1 Gather, synthesise and critically evaluate information about biological phenomena from a range of sources.
3.2 Critically analyse observations of biological phenomena by creating and developing models and/or proposing and testing hypotheses.
3.3 Design and conduct field, laboratory based, or virtual biological experiments.
3.4 Select and apply practical and/or theoretical techniques.
3.5 Collect, accurately record, interpret, analyse, and draw conclusions from biological data.

### Communication
4. Effectively synthesise and communicate biological results using a range of modes (including oral, written, and visual) for a variety of purposes and audiences.

### Personal and professional responsibility
5.1 Be accountable for their own learning and biological work by being independent and self-directed learners.
5.2 Work effectively, responsibly and safely in individual and peer or team contexts.
5.3 Demonstrate knowledge of the regulatory frameworks and ethical principles relevant to their sub-disciplinary area within biology, and apply these in practice.

### SUMMARY OF ASSESSABLE PROJECT COMPONENTS:

<table>
<thead>
<tr>
<th>Assessable Component</th>
<th>Learning Outcomes Assessed</th>
<th>Weighting</th>
<th>Due Date</th>
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</table>
| **Scientific Literature Essay** | The following LO from the lecture: Scientific literature” will be assessed:  
  - Identify a primary article and list its features  
  - Explain the differences between primary and secondary sources of scientific literature  
  - Describe the term ‘peer review’ as it applies to scientific literature  
  *Graduate Attributes developed:*  
  - Research, inquiry and analytical thinking abilities;  
  - Communication;  
  - Information literacy.  
  *Biology Threshold learning outcomes developed:*  
  4. Effectively synthesise and communicate biological results using a range of modes (including oral, written, and visual) for a variety of purposes and audiences.  
  5.1 Be accountable for their own learning and biological work by being independent and self-directed learners. | 15% | Practical 4 Week 5 |
### Graduate Attributes developed:
- Capability and motivation for intellectual development;
- Communication;
- Teamwork, collaborative and management skills; and,
- Information literacy.

### Biology Threshold learning outcomes developed:
4. Effectively synthesise and communicate biological results using a range of modes (including oral, written, and visual) for a variety of purposes and audiences.
5.2 Work effectively, responsibly and safely in individual and peer or team contexts.

<table>
<thead>
<tr>
<th>Presentation Pitch</th>
<th>5%</th>
<th>Practical 5 Week 7</th>
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<tbody>
<tr>
<td><strong>Graduate Attributes developed:</strong></td>
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<tr>
<td>• Capability and motivation for intellectual development;</td>
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<tr>
<td><strong>Biology Threshold learning outcomes developed:</strong></td>
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<tr>
<td>4. Effectively synthesise and communicate biological results using a range of modes (including oral, written, and visual) for a variety of purposes and audiences.</td>
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<tr>
<td>5.2 Work effectively, responsibly and safely in individual and peer or team contexts.</td>
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<tr>
<th>Presentation</th>
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<th>Week 9</th>
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<tbody>
<tr>
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<tr>
<td>3.1 Gather, synthesise and critically evaluate information about biological phenomena from a range of sources.</td>
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<tr>
<td>4. Effectively synthesise and communicate biological results using a range of modes (including oral, written, and visual) for a variety of purposes and audiences. 5.2 Work effectively, responsibly and safely in individual and peer or team contexts.</td>
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<tr>
<th>Portfolio</th>
<th>5%</th>
<th>Week 9</th>
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<tbody>
<tr>
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<tr>
<td>5.1 Be accountable for their own learning and biological work by being independent and self-directed learners.</td>
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<tr>
<td>5.2 Work effectively, responsibly and safely in individual and peer or team contexts.</td>
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| Total Weighting: | 40% | |

**LIST OF PROJECT TOPICS:**

After confirming the members of your Project team, you must collectively select a current biology topic for your project from the list below. These topics have been carefully selected for their alignment with core course concepts and for their feasibility for all components of this assignment. It is possible that your team may be permitted to select a topic of interest that is not on the list, but such topics must firstly be assessed and approved by your demonstrator and/or course convenors.

1. What does the koala genome tell us about the taste of eucalyptus?
2. The Tardigrade (water bear) genome has been sequenced and it’s even weirder than we thought [https://sciencealert.com/the-tardigrade-genome-has-been-sequenced-and-it-hasthe-most-foreign-dna-of-any-animal](https://sciencealert.com/the-tardigrade-genome-has-been-sequenced-and-it-hasthe-most-foreign-dna-of-any-animal)
4. The science of genetic inheritance is weirder than we thought
5. Gene-editing technique used to create low-gluten bread suitable for celiacs
6. This biohacker became the first person to edit their own DNA
   http://www.iflscience.com/health-and-medicine/this-biohacker-became-the-first-person-to-edit-his-own-dna/
7. CRISPR gene editing tool used to treat genetic disease in an animal for the first time
8. A protein found in human breast milk could help kill drug resistant bacteria
9. Do we all carry the genes for autism? http://www.sciencealert.com/we-all-carry-the-genes-for-autism-study-finds
10. The first eukaryotes without a normal cellular power supply have been found
11. Can the ‘RNA World’ be used to explain the origins of life?
12. Humans are still evolving and we can watch it happen
13. Scientists have found a woman whose eyes have a whole new type of colour receptor
    http://www.sciencealert.com/scientists-have-found-a-woman-whose-eyes-have-a-wholenew-type-of-colour-receptor
14. Scientists think they finally know why our genes are made of DNA and not RNA
15. Forget what you learned in high school – this new carbon molecule has 6 bonds
    http://www.sciencealert.com/forget-what-you-learned-in-high-school-this-new-carbon-molecule-has-6-bonds
16. We might finally know what triggered living cells to evolve for the first time
    http://www.sciencealert.com/scientists-might-have-discovered-what-allowed-life-to-evolve
17. Scientists have successfully reversed DNA aging in mice
    http://www.sciencealert.com/scientists-have-successfully-reversed-dna-aging-in-mice
18. The energy generators inside our cells reach a sizzling 50°C
19. Antibody-powered nucleic acid release using a DNA-based nanomachine
    https://www.nature.com/articles/ncomms15150
20. High intensity workouts could slow down your aging by almost a decade
21. A new study identifies 52 genes associated with human intelligence
    https://www.facebook.com/nature/posts/10154856218303167
22. The brain starts to eat itself after chronic sleep deprivation
    https://www.newscientist.com/article/2132258-the-brain-starts-to-eat-itself-after-chronicsleep-
deprivation/?utm_term=Autofeed&utm_campaign=Echobox&utm_medium=Social&cmpid
=SOC%7CNSNS%7C2017-Echobox&utm_source=Facebook#link_time=1495578558
23. CRISPR kills HIV and eats Zika 'like Pac-man'. Its next target? Cancer
https://www.wired.co.uk/article/crispr-disease-rna-hiv
24. This is the first ever nanoscale image of a living cell membrane. It's beautiful.
http://www.sciencealert.com/researchers-close-a-debate-with-a-nanoscale-image-of-
alloving-cell-membrane
25. Gastric bypass surgery gives patients a new set of helpful microbes
http://www.sciencealert.com/gastric-bypass-surgery-gives-patients-a-new-set-of-
帮助企业
26. Can gene therapy be used to switch off asthma?
http://www.sciencealert.com/genetherapy-used-to-switch-off-asthma
27. Tea consumption leads to epigenetic changes in women
28. Designer viruses successfully stimulate the immune system to fight cancer
https://www.technologynetworks.com/cancer-research/news/designer-virussuccessfully-
stimulate-the-immune-system-to-fight-cancer-289203
29. Scientists discover plants have ‘brains’ that determine when they grow
http://www.iflscience.com/plants-and-animals/scientists-discover-plants-brains-
determinegrow/
30. New semi-synthetic organism can make molecules we’ve never seen before
http://www.sciencealert.com/new-semi-synthetic-organism-can-make-molecules-we-
venever-seen-before-dna-unnatural
31. Latest DNA analysis shows the Yeti are actually just a bunch of bears
32. Babies who get more cuddles have their genetics changed for years
http://www.sciencealert.com/cuddling-babies-alters-their-genetics-dna-for-years
33. Living bacteria “From outer space” have been found on the outside of the ISS
http://www.sciencealert.com/living-bacteria-from-outer-space-found-clinging-to-iss-alienlife
34. This protein in your brain could be at the heart of creating memories
http://www.sciencealert.com/rna-binding-protein-staufen2-linked-to-synaptic-
plasticitylearning-memory
35. World’s smallest tape recorder has been built inside a living bacterium
http://www.sciencealert.com/smallest-tape-recorder-crispr-cas-bacterium

SUGGESTED
PROJECT TIMELINE

Check Moodle announcements for updates and supporting materials to guide your project.

In Lab Class Practical:

- Form project groups
- Select project topic
- Schedule a meeting time and venue for your team in Week 2/3 Individual (in own
time):
  - Work through the ‘Scientific Literature’ lesson on Moodle. Other lessons on
    conducting a literature search and referencing may also be useful. For this
    assessment if you are unfamiliar with academic writing.
  - Team members should start individually researching their topic prior to their arranged
    meeting time with their group. You need to conduct literature search (not an internet
    search) eg. using Google Scholar or PubMed. The online lesson linked to via Moodle
    on conducting a literature search may be useful here. You should identify some
    reviews related to your topic. Note: You should not pay for any of the scientific
    literature, you will be able to access most references you need for free through the
    UNSW library online.
Week 2/3  Team Meeting (out of class):
  • Scheduled meeting team meeting - ensure an agenda is distributed prior and that one
    person takes minutes (including action items) and distributes then to members shortly
    after the meeting.
  • Confirm topic and discuss scientific literature essay and assign one primary and one
    secondary research article to each team member for the purpose of completing the
    scientific literature essay. The review may be the same across members, but primary
    articles should be different.

Week 3   In Lab Class
  • Consultation with tutor: provide them with details of the initial meeting and any
    additions ones, confirm team members and topic, ask questions you may have on the
    assignment and conduction literature searches. You may also briefly discuss you
    articles with the tutor.

Week 4/5  Team Meeting (out of class):
  • Scheduled team meeting.
  • Team members should continue to research their topic, with a focus on peer-reviewed
    scientific literature (i.e. journal articles).
  • Start developing ideas for the content and format of your major presentation (see
    assignment guidelines below; presentation ‘pitch’ is due in Week 7 and final
    presentation is due in Week 9 (or 10 for Friday classes, due to the public holiday).

Week 4/5  Individual (in own time):
  • Each team member should be individually researching and writing their “scientific
    literature essay”, due in Week 5.

Week 5/6   In Lab Class
  • Consultation with tutor on progress and presentation ideas.

Week 6   Team Meeting (out of class):
  • Scheduled meeting team meeting.
  • Develop a brief 3-5 minute project ‘pitch’ that encapsulates the fundamental elements
    of your presentation plan (see below for pitch guidelines). You will pitch your
    presentation plan to the rest of your demonstrator group in the next laboratory class
    (Week 8/9)

Week 7   In Lab Class
  • Research into topic should be complete and presentation design finalised.
  • Project Presentation Pitches delivered in lab class. Your demonstrator will facilitate
    both the individual team pitches and the peer feedback sessions after each pitch.

Week 7-9  Team Meeting (out of class):
  • Scheduled meeting team meeting
  • Continue with the plan for your team presentation

Week 8   In Lab Class
  • Consultation with tutor
  • Finalise your team’s presentation and portfolio for submission Week 9 In Lab Class:
  • Project Final Presentations: Each team will have 10 minutes to showcase/highlight
    their presentation to the class.
  • Your demonstrator will help facilitate the presentations for your group.
  • Team Portfolios due to demonstrators.
In the first phase of the Project, you will be assigned to a team of approximately 4 members. As a team, you will select a current biology topic from the list provided (or an alternative current biology topic that has been approved by course convenors). The first phase of the Project will involve doing some individual research on your topic and then reporting back to your team for discussion. Your initial searches may yield different kinds of information, including news articles, popular science communications, videos and television program segments, if this assists you in understanding the topic, as well as peer-reviewed scientific journal articles.

Since scientific journal articles will be the most difficult to understand for non-experts, you may need to consult alternative sources of information until you have a basic understanding of your topic. We then encourage you to start looking at the peer-reviewed scientific literature. The Scientific Literature Essay described in this section relates directly to the peer-reviewed scientific literature that you and your team members find on your topic.

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 or literature reviews. This assignment, 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 about biology. 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, excluding references. 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 5%.

From information presented in BABS1201, you should become familiar with the different types of peerreviewed 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.

Instructions:

Your essay task is to find a REVIEW article (ie. secondary literature) 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. What is the discipline, 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.

2. 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 subdiscipline. The journal may target regional, national or international audiences.

3. Identify 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. This article should be cited in your own reference list for your essay.

4. A description of the purpose of the primary article and a brief summary of the results of the investigations that are reported within it. What was the main outcome? Why is it significant? 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. What was the aim / hypothesis investigated in the primary article? The Scientific Literature lesson on Moodle steps you through the parts of a journal article and will help you identify where to look for this. Which section did you find the aim/ hypothesis, and can your write it in your own words, suitable for a BABS1201 audience rather than an expert?

6. A sentence or two on your understanding of the nature and purpose of primary literature compared to secondary literature. Well scoring essays will be able to explain how their primary article contributed to the review article.

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 REFERENCE 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, simply reference correctly the two articles. A module on how to find and reference scientific articles correctly is available on Moodle.

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 screened for paraphrasing/plagiarism, so this is particularly important.

**Pitch – 5%**

The pitches in Week 7 give you the opportunity to describe your future plan for the presentation to your demonstrator and group to receive feedback. The pitch is a conversation, not a formal presentation, and you have five minutes to do so.

Some ideas to cover:
You need to define / describe your topic. What is it that you have chosen to present on in Weeks 9/10? If the topic has diverged somewhat from your initial article, you can describe this.
How does your topic align with the themes of the course? You will obtain feedback on whether your topic aligns well, if the material is too simple, too complex, or if you are trying to cover too much. You should also describe how you will present - as you are encouraged to engage the audience in your final presentation eg. NOT simply deliver a talk with slides (in 2018 most groups used Kahoot, but we would love you to be even more creative). You can use your pitch to get feedback on any ideas you have, and also describe how you plan to approach the rest of the project and workload allocations.

In brief, the pitch is your chance to see if you are on the 'right track' with the project. The assessment portion is there to motivate you to prepare ahead, as the pitch will be most useful if you know what you most want feedback on.

*You do not need to upload anything to Moodle associated with the presentation.*

**Presentations – 15%**

Your presentations for the project are in Weeks 9 (and Week 10 for those missing a lab due to Good Friday). The marking rubric for your presentations is available under “Assessments” and information is also provided above.

Time - You have 10 minutes, and will be stopped at that time. If you have a video that is less than five minutes in length, it is fine to briefly introduce it and simply play the video and answer questions, you do not need to take up the full 10 minutes.

Alignment with the course themes - This should have been covered in the pitch, where you were asked to state how your topic aligned with the course themes. If you received feedback on this, you must ensure this is responded to - you do not need to be explicit about how it aligns in your presentation if your demonstrator and group saw clear alignment in your pitch.

More questions? Please ask them on the Moodle forum.

*You do not need to upload anything to Moodle associated with the presentation.*

**Portfolio – 5%**

The portfolio should be compiled as you progress through the project. How you organise this is up to the group, for example, you may dedicate one member to collate the documentation every two weeks. The aim is to ensure you have clear communication between members to ensure no issues arise within the group and, if they do, you have a record of communication, inclusion and the work allocated and performed by all members.

This is the documentation of your team's process and progress towards developing the presentation. The portfolio does not have a set format, although optional templates for planning and recording meetings were provided on Moodle. You need to hand in a group submission to your demonstrator that shows how each member was included, how tasks were allocated etc.

*You do not need to upload anything to Moodle associated with the presentation.*

**D. MID TERM TEST – 15%**

The mid-term test is taken in your enrolled laboratory time in Week 6. The format is multiple choice and you are required to bring a pencil and eraser to complete the multiple choice answer sheet. You need to arrive on time as you may not be permitted late entry.

The test all material up to the end of Week 6. If you miss this assessment due to illness or misadventure, please apply for special consideration online (see page 15 for instructions).
E. FINAL THEORY EXAM – 40%

Date, Time and Venue to be advised by the exams branch.

The final examination is conducted externally during the UNSW T1 examination period. The date, time and location is determined by the exams branch and your course convenors are not permitted to communicate this information to you once the exam timetable is released (incase we make a mistake!). It is up to you to consult the exam timetable when released. The BABS1201 exam is often held across multiple locations, so we strongly advise you to consult your own timetable and not to ask a classmate.

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 the course. 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 15 for instructions).

Please note that there are NO PAST EXAM PAPERS provided in this course. To prepare, we recommend you revise the lecture notes and corresponding learning outcomes, using the quizzes, textbook, lightboard videos and any other revision activities provided for you via Moodle and your lecturers.

INTRODUCTION TO LABORATORY SAFETY

CONTENTS

1. Health & safety guidelines
2. Online Laboratory Safety Quiz
3. Meeting your classmates
4. Thinking like a scientist

OBJECTIVE

• Be aware of safe work practices in the laboratory.

Today you will complete some activities that start to develop the following Biology Threshold Learning Outcomes:

• Be accountable for their own learning and biological work by being independent and self-directed learners.
• Work effectively, responsibly and safely in individual and peer or team contexts.

UNSW Science graduate attributes:

• Capability and motivation for intellectual development.
• Ethical, social and professional understanding.

1. HEALTH & SAFETY

In your first laboratory class 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. Some basic guidelines are provided below, and a video on laboratory safety is available on Modle.

General conduct
A laboratory is intended for serious work and students are expected to behave appropriately.

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 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 assessments
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 performed in each practical class and is included in the manual. The risks are categorised as follows: Biological hazards: Such as microorganisms used in an experiment.

Chemical hazards: Most of the chemicals used in this subject are not hazardous at the concentrations that are being used, however appropriate PPE (eg. lab coat, closed shoes) should be used in the laboratory at all times. Some practicals employ hazardous chemicals. In these cases the hazard and precautions required are described in the instructions for the class, and may be updated by your demonstrator. Safety Data Sheets (SDS) are available for all of the hazardous chemicals from your demonstrator.
Physical hazards: Heat sources, such as water baths, breakable glassware, sharp objects such as plastic tips and razor blades.

Hazards involving work environments: This include ergonomics eg. the chair and desk position you use in the laboratory.

Some guidance to working safely in the laboratory is provided below. You also need to watch the video on laboratory safety before class, and your demonstrator will discuss the particular issues related to your laboratory classroom and the experiments with you.

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 for this you will be excluded from the class and marked as absent. Below are some guidelines that you must follow which will ensure good laboratory practice and minimise the consequences of risks:

Some specific guidelines:

- A laboratory coat must **ALWAYS** be worn while in the lab, and removed before leaving the laboratory. Where necessary (as advised by your demonstrator), protective gloves and safety glasses should also be worn.

- Gloves 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, nonabsorbant shoes must be worn at **ALL** times.

- Long hair must be tied back or up whilst you are in the laboratory.

- You must not eat, drink, 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.

- If there is an accident ask a fellow student to call someone in authority immediately. 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.

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

![Image of laboratory scene](https://example.com/laboratory_image)

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

2. ONLINE LABORATORY SAFETY QUIZ

In order to be permitted to take part in laboratory classes, you must also complete an online Laboratory Safety Quiz that is accessed through the BABS1201 Moodle site.

When you have finished the quiz and submitted all your answers, you will receive a mark out of 10. 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 10/10.

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.

If you have not scored 100% in the quiz you will NOT be permitted to attend lab classes until you have satisfied this requirement.

3. MEETING YOUR CLASSMATES

Team planning in a group of up to 4 (5 min)

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. The skills practised in this activity will also help you be organised and resilient at university, in the workplace and other parts of your life.

Research has shown that students that set academic goals perform better! Watch this short video on goal setting: [https://vimeo.com/120112496](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 term.
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.

4. 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:

<table>
<thead>
<tr>
<th>What sources of information do you use to help make your decisions?</th>
</tr>
</thead>
<tbody>
<tr>
<td>How do you evaluate whether these sources are reliable or not?</td>
</tr>
</tbody>
</table>
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!

SECTION 1:

PRACTICALS 1-2 EXPLORING CELL STRUCTURE

These two laboratory classes explore aspects of cell structure and concepts that will be discussed in lectures. You will also practice some of the techniques we use to explore cells.

The aims for this section are:
• Work safely in the laboratory.
• To competently use the light microscope (set up and care of the microscope).
• To report on microscopic structures observed through a light microscope (drawing observations and measuring size).
• To identify key differences between internal structures of bacterial and eukaryotic cells using the light microscope
• To competently perform basic staining procedures and light microscopy of different types of cells
• To identify an unknown cell type and describe your findings, including:
  - identifying the type of cell (bacteria, eukaryote, plant or animal);
  - describing the procedure used to identify the cells (such as the type of stain);
  - describing the characteristics of the cells that enabled you to identify them (organelles, size, and structures).

**UNSW Science graduate attributes:**

• Ethical, social and professional understanding
• Research, inquiry and analytical thinking abilities
• Communication

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**Hazards**

| Biological agent (Environmental samples – pond water) | Infection |
| Biological agent (Microorganisms, *Bacillus megaterium*) | Spills |
| Biological agent (Environmental samples – *Spirogyra*) | Cuts from broken glass |
| Razor blades, glass slides/cover slips | |

**Risks**

- Adhere to aseptic techniques.
- Notify demonstrator immediately of any spills or other incidents.
- Carry slides/cover slips in suitable containers and dispose of any broken/used items immediately in sharps containers provided. Do not use excessive force when setting up/removing slides and cover slips.
- Proper handwashing with antibacterial handwash before leaving the laboratory.

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**Controls**

- Follow demonstrator’s instructions when handling biological agents. PPE (lab coats, closed in shoes, goggles & gloves as required).
- Adhere to aseptic techniques.
- Notify demonstrator immediately of any spills or other incidents.
- Carry slides/cover slips in suitable containers and dispose of any broken/used items immediately in sharps containers provided. Do not use excessive force when setting up/removing slides and cover slips.
- Proper handwashing with antibacterial handwash before leaving the laboratory.
Electrical Equipment

- Electric shock/electrocution
- Exposure to biological agents (see above)
- Postural damage from extended periods of time working at microscope or bench

Ergonomics

- 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.

Personal Protective Equipment

- Closed in Footwear
- Lab. Coat
- Gloves

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 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.

Declaration

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

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

Student number:…………………………

PRACTICAL 1
CELL STRUCTURE I LOOKING AT CELLS USING THE LIGHT MICROSCOPE

CONTENTS

1. Light microscopy
2. Observing microscopic life
3. Structure of a bacterial cell
4. Comparison of size of eukaryotic and bacterial cells

BEFORE ATTENDING THE LABORATORY CLASS:
• Complete the Health and Safety quiz on Moodle.
• Complete Pre-lab quiz 1 on Moodle.
• Read through the notes and ensure you know how to set up a microscope.
• Watch the video on setting up a microscope.

OBJECTIVES

At the end of this practical class you should be able to:

• Work safely in the laboratory.
• To competently use the light microscope (set up and care of the microscope).
• To report on microscopic structures observed through a light microscope (drawing observations and measuring size).

1. 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.

Examples:

• 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 (µm). In other words, the smallest detail that can be seen under the highest magnification of the light microscope is 0.2 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 you will use in class.
3. Setting up the light microscope

In class video: How to set up a 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.

8. Condenser iris diaphragm adjustment

Turn the diaphragm lever (1) to vary the diaphragm opening. This allows you to adjust the contrast of your image.

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 the 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></td>
<td>Cover-slip too thick</td>
<td>Replace</td>
</tr>
</tbody>
</table>
**Unable to focus on object**

<table>
<thead>
<tr>
<th>Issue</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slide up-side down</td>
<td>Invert slide</td>
</tr>
<tr>
<td>Focusing attempts too rapid</td>
<td>Use fine focus and adjust more slowly</td>
</tr>
<tr>
<td>Objective has insufficient resolving power</td>
<td>Use higher power</td>
</tr>
<tr>
<td>Objective covered with dried immersion oil from previous use</td>
<td>Clean with lens tissue and solvent</td>
</tr>
</tbody>
</table>

**Specks in field of view**

<table>
<thead>
<tr>
<th>Issue</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dirt on eye lens of ocular</td>
<td>Clean with lens tissue</td>
</tr>
<tr>
<td>Dirt on condenser lens</td>
<td></td>
</tr>
<tr>
<td>Dirt on filter</td>
<td></td>
</tr>
</tbody>
</table>

**Moving shadows in field**

<table>
<thead>
<tr>
<th>Issue</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air and/or water bubbles in immersion oil</td>
<td>Remove oil with lens tissue. Re-apply</td>
</tr>
</tbody>
</table>

**Light suddenly reduced**

<table>
<thead>
<tr>
<th>Issue</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<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 \( \mu \)m 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 18mm.

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 0.9mm or 1mm 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>Each 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.

\[ \text{Using the scale, how high and how wide is the ‘5’?} \]
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. The image below demonstrates how this may be done by showing a bacterial cell and a scale bar indicating 500 nm.

Image: Raul Gonzalez and Cheryl Kerfeld (http://www.kerfeldlab.org/images.html) Creative Commons

2. 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. Take care not to use excessive force when setting up/removing slides and coverslips.
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.

Drawing your observations:
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. 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.

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 as an example.

![Paramecium 100x magnification](image)

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)

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:
3. **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 *Bacillus megaterium* container 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 to avoid the bacteria washing off the slide during subsequent procedures. While the slide dries, proceed to “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 6).
Note: Bacterial cells are usually either rod-shaped or round, and may form clumps or chains when growing in suspension.

Figure 6:

4. 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 measure *Euglena* or *Spirogyra* while your bacterial smear is drying.

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

*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.

1. Measure the length and width of about 10 mature *Euglena* / *Spirogyra* cells, and from these measurements, calculate the average cell length and width. Record your measurements and calculations.
2. As best as you can, estimate the average volume of a cell in the space below. Assume the ‘width’ measurement is a the diameter.

3. Using the 100X oil immersion objective, make similar measurements on the *Bacillus* cells. 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 *Euglena / Spirogyra*, calculate the average cell volume below:

---

**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.
PLEASE NOTE You must clean your lenses using the lens tissue provided. Never use ordinary tissues as these can scratch lenses.

<table>
<thead>
<tr>
<th>Biological agent</th>
<th>Infection</th>
<th>Follow demonstrators instructions when handling biological agents.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Microorganisms,</td>
<td>Spills</td>
<td>PPCE (lab coats, closed in shoes &amp; gloves as required). Adhere to aseptic techniques.</td>
</tr>
<tr>
<td><em>Rhodospirillum</em></td>
<td>Cuts from broken glass</td>
<td>Notify demonstrator immediately of any spills or other incidents.</td>
</tr>
<tr>
<td>and <em>Anabaena</em></td>
<td>Razor blade cuts</td>
<td>Carry slides/cover slips in suitable containers and dispose of any broken/used items immediately in sharps containers provided. Proper hand washing with antibacterial hand wash before leaving the laboratory.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hazardous chemicals (nitroblue tetrazolium salt)</th>
<th>Eyes: Causes irritation.</th>
<th>Personal Protection: Wear appropriate safety goggles, gloves and protective clothing.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Skin: Causes irritation.</td>
<td><strong>Eyes</strong>: Immediately flush with plenty of water for at least 15 minutes. <strong>Skin</strong>: Immediately flush area with soap and water.</td>
</tr>
<tr>
<td></td>
<td>Ingestion: Harmful if swallowed</td>
<td><strong>Label Hazard Warning</strong>: Caution! May cause eye and skin irritation. May cause respiratory and digestive tract irritation.</td>
</tr>
<tr>
<td></td>
<td>Inhalation: Causes respiratory tract irritation.</td>
<td></td>
</tr>
</tbody>
</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:……………………..

PRACTICAL 2  CELL STRUCTURE II
COMPARING CELLS USING THE LIGHT MICROSCOPE

CONTENTS

1. Staining mitochondria in eukaryotic cells
2. Staining nuclei in animal and plant cells
3. Cell walls, vacuoles & chloroplasts of eukaryotic cells
4. Other internal structures of eukaryotic cells
5. Staining bacterial cells
6. Staining of fixed cells

BEFORE ATTENDING THE LABORATORY CLASS:

• Complete Pre-lab quiz 2 on Moodle.
• Revise the content covered in your lectures on Cells (using the lecture notes, videos on Moodle and your textbook).
• If you missed Practical 1, you must read through the notes and watch the video on How to set up a microscope on Moodle.

OBJECTIVES

At the end of this practical class you should be able to:

• To identify key differences between internal structures of bacterial and eukaryotic cells using the light microscope
• To competently perform basic staining procedures and light microscopy of different types of cells
• To identify an unknown cell type and describe your findings, including:
  - identifying the type of cell (bacteria, eukaryote, plant or animal);
  - describing the procedure used to identify the cells (such as the type of stain);
  - describing the characteristics of the cells that enabled you to identify them (organelles, size, and structures).
1. **STAINING MITOCHONDRIA IN EUKARYOTIC CELLS**

Introduction:

Living cells come in a range of shapes, sizes and degrees of complexity. In this practical you will perform staining - a procedure commonly used to assist in the identification of cells. Different types of stains react with different kinds of molecules, and so cell structures may or may not stain with different stains. We can therefore use staining 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 stains in this class.

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).

- Remember from the Risk Assessment that Tetrazolium salts are hazardous!
- Ensure you wear safety glasses.
- Use the gloves provided, and when finished place them in the Scientific Waste bins.
  NEVER leave gloves, used or unused, on the bench or in non-Scientific

Procedure:

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

1. Place about enough tetrazolium solution to cover you samples (approx.. 5 drops) 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 (cutting lengthwise and longitudinal), 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).

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?*
2. 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. This step is to be performed outside the laboratory. Remove your lab coat and wash your hands before moving out into the hallway. 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.
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).

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).
4. 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>
</tr>
</thead>
</table>
4. STAINING BACTERIAL CELLS

Bacterial cells are generally smaller than eukaryotes, and have a less well defined internal structure. This makes it difficult to see them well under light microscopy, unless you use the high power (100X) oil immersion lens. Today you will look at two 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% nigrosin with a drop of *Rhodospirillum* 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 *Rhodospirillum* 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 nigrosin?

What shape are the bacteria?

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

SECTION 2:
EXPLORING CELL FUNCTION PRACTICALS
3-4

Over the next two practical classes, you will perform accurate and repeatable measurements of cell functions including:

- Osmosis and diffusion
- 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 aims for this section are:

- Calculate and report on parameters involved in diffusion through a semipermeable membrane.
• Investigate and report on the effects of osmosis on animal and plant cells.
• Use a pipettor to dispense a known volume, and make a dilution of a concentrated solution.
• 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.

**UNSW Science graduate attributes:**

• Research, inquiry and analytical thinking abilities
• Communication
• Teamwork, collaborative and management skills

---

**Hazardous chemicals (Congo Red)**

**Warning!** Causes eye irritation. May cause skin and respiratory tract irritation. Possible risk of harm to the unborn child. **May cause cancer in humans.** 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.

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

Wear gloves and safety glasses at all times when working with Congo Red.

Do not use personal items such as mobile phones or computers with gloves on.

---

**Biological hazard (horse red blood cells)**

Infection: contains animal blood product.

Handle Material as potentially infectious.
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 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:.................................

PRACTICAL 3  CELL FUNCTION I
OSMOSIS AND DIFFUSION

CONTENTS

1. Osmosis Staining mitochondria in eukaryotic cells
2. Effect of osmosis on animal and plant cells

BEFORE ATTENDING THE LABORATORY CLASS:

• Complete Pre-lab quiz 3 on Moodle.
• Revise the content covered in your lectures on Maintaining cell integrity and Nutrient and ion transport (using the lecture notes, videos on Moodle and your textbook).

OBJECTIVES

At the end of this practical class you should be able to:

• Calculate and report on parameters involved in diffusion through a semipermeable membrane.
• Investigate and report on the effects of osmosis on animal and plant cells.
• Use a pipettor to dispense a known volume, and make a dilution of a concentrated solution.

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:

- Remember from the Risk Assessment that Congo red is hazardous!
- Ensure you wear safety glasses.
- Use the gloves provided, and when finished place them in the Scientific Waste bins.
- NEVER leave gloves, used or unused, on the bench or in non-Scientific waste bins.

Work in pairs

In this part of the experiment, you will demonstrate osmosis using an artificial semipermeable 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 g Congo red in 100 mL aqueous solution).

| The molar mass of Congo Red is approximately 700 g/mol. 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.

![Figure 1.](image)

6. Note the level of the Congo red solution in the capillary tube.

7. 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).
Figure 2:
8. Use Equation 1 below to calculate the osmotic pressure exerted by the congo red solution under these circumstances. Show your calculations.

**Equation 1:** \( \Box \Box = 1000 \, RT \, (C_i - C_o) \)

Where:
- \( \Box \Box \) = 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:** \( P = \Box \Box \, gh \)

Where:
- \( \Box \Box \) = density of water (1000 kg.m\(^{-3}\)) \( P \)
- \( g \) = hydrostatic pressure (N. m\(^{-2}\)) \( g \)
- \( h \) = acceleration of gravity (9.8 m.s\(^{-2}\)) \( h \)
- \( h \) = height (m)
10. Look at the demonstration using 2% methylene 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 Methylene 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 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>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.25</td>
<td>3.75</td>
<td>50</td>
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<tr>
<td>2</td>
<td>1.50</td>
<td>3.50</td>
<td>60</td>
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<td>3</td>
<td>1.75</td>
<td>3.25</td>
<td>70</td>
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<tr>
<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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</table>
4. To each tube, add 0.1 ml (approx. 3 drops) horse 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 erythrocytes. 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>
<thead>
<tr>
<th>Solution</th>
<th>Time until complete lysis of erythrocytes</th>
</tr>
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<tbody>
<tr>
<td></td>
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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 undersurface 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>
<thead>
<tr>
<th>Tube</th>
<th>Volume of NaCl (mL)</th>
<th>Volume of distilled water (mL)</th>
<th>Final NaCl concentration (mM)</th>
</tr>
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<tbody>
<tr>
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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 coverslip.

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 plasmolyzed.

10. Record the total number of cells you count, and the number of these that are plasmolyzed. From this, calculate the percent plasmolyzed 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 plasmolyzed</th>
<th>% cells plasmolyzed</th>
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Plot the % plasmolysis versus NaCl concentration as Figure 3 below.

Figure 3:
**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 erythrocytes you investigated earlier?**

**What occupies the space between the plasmolysed protoplast and the cell wall of the Rhoeo cells?**
### 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. **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)

- **Cuts.**
- 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)

**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.

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

---

**Closed in Footwear**

**Lab. Coat**

**Gloves**

**Safety Glasses**

---

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:…………………………..

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**PRACTICAL 4**

**CELL FUNCTION II PHOTOSYNTHESIS AND RESPIRATION**
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

BEFORE ATTENDING THE LABORATORY CLASS:

• Complete Pre-lab quiz 4 on Moodle.

OBJECTIVES

At the end of this practical class you should be able to:

• 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.

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 NADP+, the light reaction can be written as:

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

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:

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

*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 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 \textit{a} to a suitable electron acceptor and the subsequent replacement of those electrons from water molecules so releasing \textit{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 H₂O 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 1.**

<table>
<thead>
<tr>
<th>Distance (cm)</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>40</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light intensity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Initial DPIP (nmol)</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>B. time for decolourisation (sec)</td>
<td></td>
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</tbody>
</table>
C. Rate of breakdown (A/B)

|        |        |        |        |

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?**
Figure 3:
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:

\[
\text{Glucose + oxygen} \xrightarrow{\text{enzymes}} \text{energy} + \text{ATP} \quad \text{lost as heat}
\]

\[
\text{carbon dioxide} + \text{water}
\]

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:

Work in groups of 3.

- Soda lime is a strong alkali. Do not get in on skin or clothing.
- Ensure you wear safety glasses and gloves.
If some spills on your skin or clothing, tell your demonstrator immediately and wash the affected area for several minutes with running water.

1. Fill a test tube with germinated mung beans.
2. Put soda lime in the small wire basket and place it in the test tube, making sure that it is not touching the beans.
3. Using the Figures 4 and 5 as a guide, construct your respirometer as follows:

![Figure 4: Respirometer design.](image)

![Figure 5: Respirometer design.](image)
4. Dampen the sides of the rubber bung with water and insert it with its plastic tube firmly into the test tube.

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

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

7. 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.

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

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

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

11. 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.

12. 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.

13. Analyse your results as follows:

14. 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.

15. 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.

16. 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>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
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<tr>
<td>15</td>
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<tr>
<td>20</td>
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<tr>
<td>25</td>
<td></td>
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<td>30</td>
<td></td>
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<tr>
<td>35</td>
<td></td>
<td></td>
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<tr>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 6:
SECTION 3: EXPLORING GENES
PRACTICALS 5 - 6

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 two 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:

• Describe human karyotyping and its role in ascertaining sex and detecting chromosomal abnormality.

• List and explain the stages in mitosis and meiosis.

• Compare and contrast meiosis and mitosis.

• List the reagents needed in a PCR and explain their purpose.

• Interpret the results of a PCR experiment, and construct a pedigree from those results.

UNSW Science graduate attributes:

• Research, inquiry and analytical thinking abilities
• Ethical, social and professional understanding
• Teamwork, collaborative and management skills
### General

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

### Physical injury (razor blades, needles)

| Cuts | Do not walk around laboratory with exposed sharps. Dispose of all used blades in the sharps bins provided on your bench. |

### Hazardous chemicals (45% acetic acid mounting medium, 70% ethanol)

| Do not get in on skin or clothing. If you do, wash the affected area for several minutes with running water. | Wear **Personal protective equipment** (gowns, gloves and enclosed shoes). Wash hands before leaving the laboratory. Wear safety glasses when working with concentrated acid. |

| Wear gloves at all times. |

---

**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.

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.

---

**Declaration**

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

Signature:____________________________________________________Date:______________________

Student number:_____________________

---

**PRACTICAL 5 GENES I**
MITOSIS AND MEIOSIS

CONTENTS
1. Observing karyotypes
2. Mitosis in the onion root tip
3. Meiosis
4. Setting up a PCR

BEFORE ATTENDING THE LABORATORY CLASS:

• Complete Pre-lab quiz 5 on Moodle.

OBJECTIVES

• Describe human karyotyping and its role in ascertaining sex and detecting chromosomal abnormality.
• List and explain the stages in mitosis and meiosis.
• Compare and contrast meiosis and mitosis.
• List the reagents needed in a PCR and explain their purpose.
• Interpret the results of a PCR experiment, and construct a pedigree from those results.
• 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 second law).
• Verify that the law of independent assortment holds true for alleles in a dihybrid cross between two heterozygous individuals.
• Observe genomic DNA and its properties.

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.
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:

<table>
<thead>
<tr>
<th>Group</th>
<th>Chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1-3</td>
</tr>
<tr>
<td>C</td>
<td>6-12</td>
</tr>
<tr>
<td>E</td>
<td>16-18</td>
</tr>
<tr>
<td>G</td>
<td>21 and 22</td>
</tr>
<tr>
<td>B</td>
<td>4 and 5</td>
</tr>
<tr>
<td>D</td>
<td>13-15</td>
</tr>
<tr>
<td>F</td>
<td>19 and 20</td>
</tr>
<tr>
<td></td>
<td>Sex chromosomes X and Y</td>
</tr>
</tbody>
</table>

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 chromatinstaining 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

1. Metacentric
2. Submetacentric
3. Acrocentric
4. Telocentric

Figure 3: Centromere locations

Observing HeLa cell karyotypes

Your aim is to prepare and analyse metaphase chromosome spreads (Figure 3). 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.

Figure 3: HeLa cell metaphase chromosome spread
Procedure:

Work in pairs.

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?

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**

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).

*Define following terms:*

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>diploid</td>
<td></td>
</tr>
<tr>
<td>haploid</td>
<td></td>
</tr>
<tr>
<td>homologous chromosomes</td>
<td></td>
</tr>
<tr>
<td>locus</td>
<td></td>
</tr>
<tr>
<td>heterozygous</td>
<td></td>
</tr>
<tr>
<td>homozygous</td>
<td></td>
</tr>
<tr>
<td>dominant</td>
<td></td>
</tr>
<tr>
<td>recessive</td>
<td></td>
</tr>
</tbody>
</table>
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.

Figure 5: Overview of meiosis. In this example, diploids have 2n=2 chromosomes.

**How many cells form during the process of meiosis?**
Are the cells formed in meiosis haploid (n) or diploid (2n)?

If the same set of chromosomes shown above were to undergo mitosis, would the resulting cells be haploid or diploid?

List three major differences between meiosis and mitosis:
1.
2.
3.

3. SETTING UP A 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.

Background:

PCR is a widely used technique in molecular biology that enables amplification (copying) 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 $3 \times 10^9$ bps of DNA), a unique sequence of 300 bp comprises 0.1 µg 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.
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.

---

### Hazards

<table>
<thead>
<tr>
<th>General</th>
<th>See below</th>
<th><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.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sharps</td>
<td>Physical injury</td>
<td>Use caution working with pipette tips. Dispose of used tips in the sharps bins on your bench.</td>
</tr>
</tbody>
</table>
### Use of electrical equipment

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Hazard</th>
<th>Precaution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrocution</td>
<td>Fire</td>
<td>Electrocution, fire, tripping over cables</td>
</tr>
<tr>
<td></td>
<td></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>Chemicals (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</td>
<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></td>
<td></td>
<td>Wear Personal protective equipment (gowns, gloves and enclosed shoes). Wash hands before leaving the laboratory. Wear gloves at all times.</td>
</tr>
<tr>
<td></td>
<td>UV light (Gel doc transilluminator)</td>
<td>UV damage to skin and/or eyes</td>
</tr>
</tbody>
</table>

---

**Personal Protective Equipment**

- Closed in Footwear
- Lab. Coat
- Gloves

---

**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 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:.................................

---

**PRACTICAL 6 GENES II**

**GENETIC INHERITANCE**

---

**CONTENTS**

1. Inheritance and Mendel’s laws
2. Investigating human pedigrees
3. Completion and analysis of PCR screening
4. Precipitation of genomic DNA from strawberries
BEFORE ATTENDING THE LABORATORY CLASS:

• Complete Pre-lab quiz 6 on Moodle.

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 second law).
• Verify that the law of independent assortment holds true for alleles in a dihybrid cross between two heterozygous individuals.
• Interpret the results of a PCR experiment, and construct a pedigree from those results.
• Observe genomic DNA and its properties.

1. INHERITANCE 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?
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></th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>VV (full)</td>
</tr>
<tr>
<td>Vv (full)</td>
<td></td>
</tr>
</tbody>
</table>
Gametes from father

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

| Phenotype | Proportion |
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>
<td></td>
<td></td>
</tr>
<tr>
<td>Class total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*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 2).
Figure 2: 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 *F*$_1$ generation by filling in the Punnett square below.

**Gametes from father**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Gametes from mother**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</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>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
</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:

<table>
<thead>
<tr>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>full wing, red eye</td>
</tr>
<tr>
<td>full wing, white eye</td>
</tr>
<tr>
<td>short wing, red eye</td>
</tr>
<tr>
<td>short wing, white eye</td>
</tr>
</tbody>
</table>

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>Phenotype</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>
</tbody>
</table>
Do your results support what you predicted from the Punnett square? Explain:
2. INVESTIGATING HUMAN PEDIGREES

Introduction

Once you have the data from your PCR experiment, 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.

![Pedigree Diagram]

Figure 3: Example pedigree.

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 3?

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&lt;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&gt;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 vision deficiency:

If you consent, your demonstrators will test you for colour vision deficiency, a fairly common genetic variant. Record the information below:

Table 2:

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number tested</td>
<td></td>
</tr>
<tr>
<td>Number with colour vision deficiency</td>
<td></td>
</tr>
<tr>
<td>Incidence of colour vision deficiency (% total tested)</td>
<td></td>
</tr>
<tr>
<td>Proportion of total colour vision deficiency individuals</td>
<td></td>
</tr>
</tbody>
</table>
Can you speculate as to the mode of transmission of colour vision deficiency? 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 3, 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>Trait</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:

3. COMPLETION AND ANALYSIS OF PCR SCREENING

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. 4 mm 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 100 ng) 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 220 V for 15 mins) 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.

What is the mode of transmission of Duchenne’s Muscular Dystrophy?

4. PRECIPITATION OF GENOMIC DNA FROM STRAWBERRIES

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 strawberries 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?**