## CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Welcome</td>
<td>2</td>
</tr>
<tr>
<td>Quick Answers to BABS1201 FAQs</td>
<td>3</td>
</tr>
<tr>
<td>BABS1201 Weekly Class and Assessment Schedule</td>
<td>5</td>
</tr>
<tr>
<td>Course Identity</td>
<td>6</td>
</tr>
<tr>
<td>Course Resources</td>
<td>7</td>
</tr>
<tr>
<td>Course Assessment Schedule and Summary</td>
<td>8</td>
</tr>
<tr>
<td>Course Outline</td>
<td>10</td>
</tr>
<tr>
<td>Course Structure</td>
<td>11</td>
</tr>
<tr>
<td>Graduate Attributes</td>
<td>12</td>
</tr>
<tr>
<td>Lecture Program</td>
<td>13</td>
</tr>
<tr>
<td>Administrative Matters</td>
<td>17</td>
</tr>
<tr>
<td><strong>Special Consideration and Further Assessment</strong></td>
<td>18</td>
</tr>
<tr>
<td>Academic Honesty and Plagiarism</td>
<td>19</td>
</tr>
<tr>
<td>Course Assessment – Detailed instructions</td>
<td>20</td>
</tr>
<tr>
<td>- Online laboratory safety quiz</td>
<td>20</td>
</tr>
<tr>
<td>- Mid-session tests I and II</td>
<td>21</td>
</tr>
<tr>
<td>- Science communication project</td>
<td>22</td>
</tr>
<tr>
<td>- Scientific literature essay</td>
<td>30</td>
</tr>
<tr>
<td>- Mastering biology and final exam</td>
<td>32</td>
</tr>
<tr>
<td>Introduction to Laboratory Safety</td>
<td>33</td>
</tr>
<tr>
<td>Practical 1: Cell Structure I - Looking at cells using the light microscope</td>
<td>49</td>
</tr>
<tr>
<td>Practical 2: Cell Structure II - Comparing cells using the light microscope</td>
<td>56</td>
</tr>
<tr>
<td>Practical 3: Cell Function I - Osmosis and diffusion</td>
<td>70</td>
</tr>
<tr>
<td>Practical 4: Cell Function II - Photosynthesis and respiration</td>
<td>85</td>
</tr>
<tr>
<td>Practical 5: Genes I – Mitosis and meiosis</td>
<td>101</td>
</tr>
<tr>
<td>Practical 6: Genes II – Genetic inheritance</td>
<td>117</td>
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</table>
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, before Practical 1, 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 will be posted on Moodle as the due dates approach. It is your responsibility to ensure you can access the Mastering Biology resources via Moodle well before your first quiz.

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

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

We look forward to teaching you.

Rebecca and John
Course Convenors
GENERAL

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

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

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

3. Post your question to the 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.

• 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 Mastering Biology revision materials available via Moodle.

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 beyond 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 Mastering Biology activities are particularly useful for exam preparation.

ABSENCES AND ASSESSMENTS

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

Attendance at all lab classes is compulsory. If you miss a lab class due to illness or some other unavoidable circumstance that can be explained using professional documentation (e.g. a medical certificate), you should provide your demonstrator with this documentation the following week.
• I missed the mid-session exam. What should I do?

If you miss the mid-session exam due to illness or some other unavoidable circumstance that can be explained using professional documentation (e.g. a medical certificate), you should apply for Special Consideration online (see page 18 for instructions). You will be notified of the outcome of your application before the end of session through myUNSW and/or your UNSW email account.

• I could not submit my assessment on time. What should I do?

If you cannot submit your essay or practical report by the due date due to illness or an unavoidable circumstance that can be explained using professional documentation (e.g. a medical certificate), you should apply for Special Consideration online (see page 18 for instructions) and submit the assessment item as soon as possible. You will be notified of the outcome of your application before the end of session through myUNSW and/or your UNSW email account. If you do not submit an assessment item by the submission deadline and you do not have a valid excuse, the appropriate late penalties will be applied to your final mark for that assessment item.

• I missed (or was sick during) the final exam. What should I do?

If you miss the final exam due to illness or some other unavoidable circumstance that can be explained using professional documentation (e.g. a medical certificate), you should apply for Special Consideration online (see page 18 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 18 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 18 for details of the supplementary exam.

Please note that if you are offered a supplementary exam, you will only be given one opportunity to attend this, unless there are exceptional circumstances.
<table>
<thead>
<tr>
<th>Week No.</th>
<th>Week Commencing</th>
<th>LECTURE A</th>
<th>LECTURE B</th>
<th>Laboratory (even weeks)</th>
<th>Laboratory (odd weeks)</th>
<th>Assessments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26-Feb</td>
<td>1. Course introduction – RLB/JW</td>
<td>2. Cells I - RLB</td>
<td>Introduction to laboratory safety*</td>
<td>Introduction to laboratory safety*</td>
<td>Online lab safety quiz</td>
</tr>
<tr>
<td>2</td>
<td>5-Mar</td>
<td>3. Cells II - RLB</td>
<td>4. Macromolecules I (CHO &amp; fats) - RLB</td>
<td>Prac 1 Cell structure I</td>
<td>Scientific literature module online**</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>12-Mar</td>
<td>5. Macromolecules II (Proteins) - RLB</td>
<td>6. Macromolecules III (DNA) - LLM</td>
<td>Scientific literature module online**</td>
<td>Prac 1 Cell structure II</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>26-Mar</td>
<td>MID-SESSION TEST I</td>
<td>PUBLIC HOLIDAY</td>
<td>Prac 2 Cell Structure II</td>
<td>-</td>
<td>Mid-session test 1 (10%)</td>
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<tr>
<td></td>
<td>2-Apr</td>
<td>MID-SESSION BREAK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>9-Apr</td>
<td>9. Metabolism I – JW</td>
<td>10. Metabolism II - JW</td>
<td>Prac 3 Cell function I</td>
<td></td>
<td>Essay due Prac 3 (10%)</td>
</tr>
<tr>
<td>7</td>
<td>16-Apr</td>
<td>11. Photosynthesis - RLB</td>
<td>12. DNA Replication - LLM</td>
<td>Prac 3 Cell function I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>30-Apr</td>
<td>15. Gene expression II - LLM</td>
<td>16. Polymerase Chain Reaction - LLM</td>
<td>Prac 4 Cell function II</td>
<td></td>
<td>Mastering biology Quiz 3 (1%)</td>
</tr>
<tr>
<td>10</td>
<td>7-May</td>
<td>MID-SESSION TEST II</td>
<td>17. Mutation - AMG</td>
<td>Prac 5 Genes I</td>
<td></td>
<td>Mastering biology Quiz 4 (1%)</td>
</tr>
<tr>
<td>13</td>
<td>28-May</td>
<td>No lecture</td>
<td>No lecture</td>
<td>-</td>
<td>Prac 6 Genes II</td>
<td>Mastering biology Quiz 5 (1%)</td>
</tr>
</tbody>
</table>

JW: John Wilson, RLB: Rebecca LeBard, LLM: Louise Lutze-Mann, VS: Vladimir Sytnyk, PW: Paul Waters

*Introduction to laboratory safety can be found on page 33 of your Course Manual and is to be completed outside of class hours.

** The ‘Scientific Literature’ module is found on Moodle and is to be completed outside of class hours.
<table>
<thead>
<tr>
<th>Course Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Course Code</strong></td>
</tr>
<tr>
<td><strong>Course Name</strong></td>
</tr>
<tr>
<td><strong>Academic Unit</strong></td>
</tr>
<tr>
<td><strong>Level of Course</strong></td>
</tr>
<tr>
<td><strong>Units of Credit</strong></td>
</tr>
<tr>
<td><strong>Assumed Knowledge, Prerequisites or Co-requisites</strong></td>
</tr>
<tr>
<td><strong>Hours per Week</strong></td>
</tr>
<tr>
<td><strong>Number of Weeks</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Course Convenors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dr Rebecca LeBard</strong></td>
</tr>
<tr>
<td>Room 103, Level 1, Biological Sciences Building (eastern end)</td>
</tr>
<tr>
<td>t (02) 9385 2026; Meetings by appointment only.</td>
</tr>
<tr>
<td>Email: <a href="mailto:r.lebard@unsw.edu.au">r.lebard@unsw.edu.au</a></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>John Wilson</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room G27 (inside the BSB Office), Biological Sciences Building</td>
</tr>
<tr>
<td>t (02) 9385 8156; Meetings by appointment only.</td>
</tr>
<tr>
<td>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>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sally Crane</strong></td>
</tr>
<tr>
<td>BABS1201 Course Administrator</td>
</tr>
<tr>
<td>E-mail: <a href="mailto:BABS1201@unsw.edu.au">BABS1201@unsw.edu.au</a></td>
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</table>

<table>
<thead>
<tr>
<th>BABS/SOMS/BEES (BSB) Student office</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room G27, Ground floor, Biological Sciences Building</td>
</tr>
<tr>
<td>t (02) 9385 8047; Opening Hours: 9:00am – 4:30pm, Mon-Fri.</td>
</tr>
<tr>
<td>Email: <a href="mailto:BABSStudent@unsw.edu.au">BABSStudent@unsw.edu.au</a></td>
</tr>
</tbody>
</table>
# Course Resources

This textbook complements the Mastering biology learning resources available to you throughout the course accessible from the course Moodle site.  
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 an on-line course management software. This website contains background information, links to resources, lecture notes, and discussion forums. Once you are enrolled in BABS1201, you can access the Moodle site at: [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.  
**Please Note:** If you choose to print your own copy, make sure that you bind it or file it in a folder and bring the COMPLETE manual to all lab classes. |
| Practical Class Requirements | For all practical classes students are required to bring:  
- The complete BABS1201 Molecules, Cells & Genes Course Manual. Read the instructions in advance for each practical.  
- A laboratory coat and closed shoes. This is required by Health and Safety (HS) regulations, and you will not be permitted to participate in practicals if you are inappropriately clothed. If you have long hair, you must also wear it tied back during practical classes. Laboratory coats can be purchased from various stores on campus.  
- Material for recording your observations and findings as appropriate for each class. These items include: a pen, an HB pencil, eraser, ruler and lined paper for written observations and plain paper for drawings. |
<table>
<thead>
<tr>
<th>Assessment</th>
<th>Brief Description</th>
<th>Due Date</th>
<th>Weight</th>
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</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% BEFORE your first practical class.</td>
<td>Before Practical 1</td>
<td>N/A</td>
</tr>
<tr>
<td>B. Session Tests I and II</td>
<td><strong>TEST 1</strong> Duration: 40 minutes. <strong>Format:</strong> multiple choice questions. <strong>Content:</strong> all theory and practical material from Weeks 1-4 (inclusive). <strong>Venue:</strong> online <strong>Time:</strong> your enrolled lecture time</td>
<td>Week 5</td>
<td>20%</td>
</tr>
<tr>
<td></td>
<td><strong>TEST 2</strong> Duration: 40 minutes. <strong>Format:</strong> multiple choice questions. <strong>Content:</strong> all theory and practical material from Weeks 6-9 (inclusive). <strong>Venue:</strong> online <strong>Time:</strong> your enrolled lecture time</td>
<td>Week 10</td>
<td></td>
</tr>
<tr>
<td>C. Science Communication Project</td>
<td><strong>ESSAY.</strong> You must prepare and submit your INDIVIDUAL essay in class (hard copy) and online via the Turnitin plagiarism checking software in Moodle. Check Moodle announcements regularly for any changes.</td>
<td>Practical 3 Week 6/7</td>
<td>10%</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 4 Week 8/9</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 advanced biology topic for submission and showcasing at the end of session</td>
<td>Practical 6 Week 12/13</td>
<td>10%</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>Practical 6 Week 12/13</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td><strong>REFLECTIVE SUMMARY.</strong> An INDIVIDUAL describes your personal learning from both positive and negative experience throughout the duration of the Project, including a peer evaluation of team member contributions to all components of the project, and how any problems could have been better managed by the team.</td>
<td>Practical 6 Week 12/13</td>
<td>5%</td>
</tr>
<tr>
<td>D. Mastering Biology</td>
<td>Online Mastering biology tasks designed to provide you with formative feedback on how you are progressing in the course. Each task is a short quiz (15-30 minutes) worth 1% of your final assessment for the course. It is highly recommended that you at least complete the online activities detailed in the manual in the weeks you do not have a laboratory class in preparation for these quizzes.</td>
<td>11:59pm Friday Weeks 4, 8, 9, 10 and 13</td>
<td>5%</td>
</tr>
<tr>
<td>H. 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 1-13 of session.</td>
<td>June (Date to be advised by exams branch)</td>
<td>40%</td>
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</table>
# Mastering Biology Assessment Schedule and Summary

<table>
<thead>
<tr>
<th>Quiz</th>
<th>Topic</th>
<th>Median Time</th>
<th>Date Available Online</th>
<th>Date Due</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quiz 1: Prokaryotic Versus Eukaryotic Cell Structure</td>
<td></td>
<td></td>
<td>Week 3 12 March</td>
<td>Week 4 23 March</td>
</tr>
<tr>
<td>Activity: Prokaryotic Cell Structure and Function</td>
<td></td>
<td>5 min</td>
<td></td>
<td></td>
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<tr>
<td>Activity: Review: Animal Cell Structure and Function</td>
<td></td>
<td>9 min</td>
<td></td>
<td></td>
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<tr>
<td>Total time</td>
<td></td>
<td>14 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quiz 2: Osmosis and Diffusion</td>
<td></td>
<td></td>
<td>Week 7 16 April</td>
<td>Week 8 27 April</td>
</tr>
<tr>
<td>Activity: Selective Permeability of Membranes</td>
<td></td>
<td>2 min</td>
<td></td>
<td></td>
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<tr>
<td>Activity: Diffusion</td>
<td></td>
<td>1 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity: Diffusion and Osmosis</td>
<td></td>
<td>6 min</td>
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<td></td>
</tr>
<tr>
<td>Activity: Facilitated Diffusion</td>
<td></td>
<td>1 min</td>
<td></td>
<td></td>
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<tr>
<td>Activity: Osmosis and Water Balance in Cells</td>
<td></td>
<td>4 min</td>
<td></td>
<td></td>
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<tr>
<td>Activity: Active Transport</td>
<td></td>
<td>2 min</td>
<td></td>
<td></td>
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<tr>
<td>Total time</td>
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<td>15 min</td>
<td></td>
<td></td>
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<td>Quiz 3: Photosynthesis</td>
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<td></td>
<td>Week 8 23 April</td>
<td>Week 9 4 May</td>
</tr>
<tr>
<td>Activity: Overview of Photosynthesis</td>
<td></td>
<td>3 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity: The Sites of Photosynthesis</td>
<td></td>
<td>1 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tutorial: Energy Flow in Plants - Concept Map</td>
<td></td>
<td>3 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity: The Light Reactions</td>
<td></td>
<td>4 min</td>
<td></td>
<td></td>
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<tr>
<td>Activity: Photosynthesis</td>
<td></td>
<td>5 min</td>
<td></td>
<td></td>
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<tr>
<td>Activity: The Calvin Cycle</td>
<td></td>
<td>3 min</td>
<td></td>
<td></td>
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<tr>
<td>Total time</td>
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<td>19 min</td>
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<tr>
<td>Quiz 4: Mitosis and Meiosis</td>
<td></td>
<td></td>
<td>Week 9 30 April</td>
<td>Week 10 11 May</td>
</tr>
<tr>
<td>Activity: Mitosis and Cytokinesis Animation</td>
<td></td>
<td>8 min</td>
<td></td>
<td></td>
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<tr>
<td>Activity: The Cell Cycle</td>
<td></td>
<td>3 min</td>
<td></td>
<td></td>
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<tr>
<td>Activity: Meiosis Animation</td>
<td></td>
<td>10 min</td>
<td></td>
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<tr>
<td>Total time</td>
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<tr>
<td>Quiz 5: DNA Structure and Replication</td>
<td></td>
<td></td>
<td>Week 12 21 May</td>
<td>Week 13 1 June</td>
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<tr>
<td>Activity: DNA and RNA Structure</td>
<td></td>
<td>8 min</td>
<td></td>
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<tr>
<td>Activity: DNA Replication: A Closer Look</td>
<td></td>
<td>4 min</td>
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<tr>
<td>Tutorial: DNA Replication</td>
<td></td>
<td>2 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity: DNA Replication: A Review</td>
<td></td>
<td>4 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity: DNA Replication: An Overview</td>
<td></td>
<td>3 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity: DNA Synthesis</td>
<td></td>
<td>4 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total time</td>
<td></td>
<td>25 min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Each quiz is worth 1% of your final assessment (5% total).*

Quizzes are not timed, but each must be completed by the due date.
<table>
<thead>
<tr>
<th>Course Outline</th>
</tr>
</thead>
<tbody>
<tr>
<td>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.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Theme 1: Thinking like a scientist</th>
</tr>
</thead>
<tbody>
<tr>
<td>This theme introduces the skills of scientific thinking, including how to decide what is true or plausible, and how scientists communicate. It also exposes you to cutting edge research being conducted at UNSW. Lectures and practical classes on this theme are interspersed through the session, enabling you:</td>
</tr>
<tr>
<td>To comprehend that science is a never-ending exploration, and that knowledge is provisional.</td>
</tr>
<tr>
<td>To identify the principal characteristics of scientific evidence.</td>
</tr>
<tr>
<td>To understand how scientists approach the investigation of a topic.</td>
</tr>
<tr>
<td>To communicate the principles of scientific findings to other scientists.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Theme 2: Cell biology and cell architecture</th>
</tr>
</thead>
<tbody>
<tr>
<td>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:</td>
</tr>
<tr>
<td>To understand the evolutionary origins of life, and of the diversity of life.</td>
</tr>
<tr>
<td>To identify the different types of living cells, and the main similarities and differences between them.</td>
</tr>
<tr>
<td>To explain how different cell types are identified.</td>
</tr>
<tr>
<td>To describe important cell structures and relate these to function.</td>
</tr>
<tr>
<td>To compare and contrast cell structures in eukaryotes and bacteria.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Theme 3: Metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>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:</td>
</tr>
<tr>
<td>To describe the essential differences between proteins, carbohydrates and lipids.</td>
</tr>
<tr>
<td>To describe the processes by which these molecules enter cells.</td>
</tr>
<tr>
<td>To comprehend the processes of generating energy for cellular function.</td>
</tr>
<tr>
<td>To compare and contrast energy generation in animals and plants.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Theme 4: Genetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>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:</td>
</tr>
<tr>
<td>To describe the essential structures of genetic material (nucleic acids, genes, chromosomes).</td>
</tr>
<tr>
<td>To explain the processes by which cells divide.</td>
</tr>
<tr>
<td>To describe the principal steps in the control of gene expression and the production of functional proteins.</td>
</tr>
<tr>
<td>To relate these structures and processes to the inheritance of genetic characteristics.</td>
</tr>
<tr>
<td>To explain the uses of recombinant DNA technology in at least one situation relating to investigation of gene function.</td>
</tr>
</tbody>
</table>
## 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 session on laboratory safety and procedures in Week 1. Should you be unable to attend your practical class for any reason, you will not be able to do “make-up” labs. For unavoidable absences from practical classes, you must provide your demonstrator with a medical certificate or other professional documentation that supports the reason for your absence. See FAQ on page 3 and Expectations of Students on page 17 for details on absences from classes.

### Lectures

Lectures emphasise certain principles covered in the text, provide an overview, and connect the individual components of the course. The lectures also serve to update and extend text coverage, using examples from current research. All of the lecturers in Molecules, Cells and Genes are active in research and have well-established reputations in the fields in which they teach. At UNSW, the people who teach you biology have made significant contributions to your area of study.

Unlike high school, we do not take roll at lectures, and there is no compulsion to attend. There are often ideas or concepts covered in lectures that may not necessarily be dealt with in your text. Lectures also serve to highlight areas that we believe you should focus on in the textbook.

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.

Please be aware that whilst the lecture notes are comprehensive, they are no substitute for taking your own additional notes based on the lectures and textbook material.
### 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></td>
<td></td>
<td></td>
<td>3</td>
<td>Guided laboratory practicals; independent and collaborative lab research; and independent research.</td>
</tr>
<tr>
<td>Capability and motivation for intellectual development</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>Small group discussions; essay and project presentation; Mastering biology online resources.</td>
</tr>
<tr>
<td>Ethical, social and professional understanding</td>
<td></td>
<td></td>
<td></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>
</tr>
<tr>
<td>Communication</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>Development of scientific writing skills through introduction to scientific literature (essay); and the project.</td>
</tr>
<tr>
<td>Teamwork, collaborative and management skills</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>Team independent research project; facilitation of group discussions in class and on Moodle.</td>
</tr>
<tr>
<td>Information literacy</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>Introduction to finding reviews and primary scientific literature (essay), team project.</td>
</tr>
<tr>
<td><strong>Teaching strategies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>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 (<a href="http://www.guidelinesonlearning.unsw.edu.au">www.guidelinesonlearning.unsw.edu.au</a>) to:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>o Teach students the process of scientific inquiry through progressive cycles of critical analysis of their research and their own thinking;</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>o Facilitate multidisciplinary thinking to reflect current research and professional practice in the sciences;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>o Reinforce deep learning and promote collaborative inquiry;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>o Integrate students’ disciplinary understanding and research practice with the development of their communication skills, teamwork, and information literacy skills.</td>
</tr>
<tr>
<td>Lecture Program</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>----------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Please note that the lecture topics and learning outcomes listed below are a guide only. Individual lecturers may provide you with updated topics and learning outcomes.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>What is BABS1201?</th>
<th>Lecture topics</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Course introduction.</td>
<td></td>
</tr>
<tr>
<td>• Use of course resources: lectures, Moodle, and practicals.</td>
<td></td>
</tr>
<tr>
<td>• Assessments.</td>
<td></td>
</tr>
<tr>
<td>• How to study and excel in your assessments.</td>
<td></td>
</tr>
<tr>
<td>• Plagiarism.</td>
<td></td>
</tr>
</tbody>
</table>

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

**Learning outcomes**
- To understand the characteristics of life.
- To be able to identify the fundamental differences between bacteria and eukaryotic cells.
- To describe the concept of endosymbiosis.

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

**Learning outcomes**
- To identify characteristic structures of eukaryotic and bacterial cells and to describe their basic functions.
- To describe the endomembrane system.
- To list the main components of the cytoskeleton and briefly describe their roles in the cell.

<table>
<thead>
<tr>
<th>Macromolecules I</th>
<th>Lecture topics</th>
</tr>
</thead>
<tbody>
<tr>
<td>• The four main organic components of cells – nucleic acids, proteins, lipids, carbohydrates.</td>
<td></td>
</tr>
<tr>
<td>• Identification of important similarities &amp; differences in macromolecular structure.</td>
<td></td>
</tr>
<tr>
<td>• Introductory concepts of breakdown and synthesis.</td>
<td></td>
</tr>
<tr>
<td>• Functions of macromolecules – structural, food storage, enzymes.</td>
<td></td>
</tr>
</tbody>
</table>

**Learning outcomes**
- To identify characteristic structures of protein, carbohydrate and lipid molecules.
- To describe the principal elements of their formation from and breakdown to their molecular subunits.
- To identify the importance of these molecules in cell structure and in nutrition.

<table>
<thead>
<tr>
<th>Macromolecules III (DNA)</th>
<th>Lecture topics</th>
</tr>
</thead>
<tbody>
<tr>
<td>• What is the genetic information?</td>
<td></td>
</tr>
<tr>
<td>• An introduction to nucleic acid structure.</td>
<td></td>
</tr>
<tr>
<td>• Bases as code.</td>
<td></td>
</tr>
</tbody>
</table>

**Learning outcomes**
- Describe the basic structure of nucleic acids.
- Explain how genetic information is encoded in nucleic acids.
- Identify the differences between DNA & RNA.
Please note that the lecture topics and learning outcomes listed below are a guide only. Individual lecturers may provide you with updated topics and learning outcomes.

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

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

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

<table>
<thead>
<tr>
<th>Metabolism II: Extracting energy from food</th>
<th><strong>Lecture topics</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overview of the catabolism of carbohydrate, fat and protein.</td>
</tr>
<tr>
<td></td>
<td>Overview of glycolysis, the TCA cycle and the respiratory chain.</td>
</tr>
<tr>
<td></td>
<td>Chemiosmosis - the formation of ATP by oxidative phosphorylation.</td>
</tr>
<tr>
<td></td>
<td>ATP yields from glucose catabolism.</td>
</tr>
<tr>
<td><strong>Learning outcomes</strong></td>
<td>Describe the convergent catabolism of different macromolecules.</td>
</tr>
<tr>
<td></td>
<td>Describe the central features of glycolysis, the TCA cycle and oxidative phosphorylation.</td>
</tr>
<tr>
<td></td>
<td>To comprehend the basic principles of chemiosmosis - the generation of a proton gradient by, for example, the respiratory chain and the utilisation of the gradient by ATP synthase.</td>
</tr>
<tr>
<td></td>
<td>To explain the advantages of respiration over fermentation with respect to energy yields.</td>
</tr>
<tr>
<td>Photosynthesis: Synthesising food from energy</td>
<td>Lecture topics</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td></td>
<td>• Overview of photosynthesis.</td>
</tr>
<tr>
<td></td>
<td>• Light harvesting.</td>
</tr>
<tr>
<td></td>
<td>• The light reactions of photosynthesis.</td>
</tr>
<tr>
<td></td>
<td>• The Calvin cycle.</td>
</tr>
<tr>
<td>Learning outcomes</td>
<td>• To explain the functions of the different stages in photosynthesis - light harvesting, the conversion of light energy into chemical energy and carbon dioxide fixation.</td>
</tr>
<tr>
<td></td>
<td>• To explain the overall organisation of the light reactions in photosystems I and II.</td>
</tr>
<tr>
<td></td>
<td>• To outline the fixation of carbon dioxide and synthesis of glucose in the Calvin cycle.</td>
</tr>
<tr>
<td></td>
<td>• To compare and contrast the generation of energy from photosynthesis and oxidation.</td>
</tr>
<tr>
<td>DNA replication</td>
<td>Lecture topics</td>
</tr>
<tr>
<td></td>
<td>• Mechanisms of DNA synthesis in bacteria and eukaryotes.</td>
</tr>
<tr>
<td>Learning outcomes</td>
<td>• Explain the semi-conservative model of DNA replication.</td>
</tr>
<tr>
<td></td>
<td>• Describe the basic steps involved in the process of DNA replication.</td>
</tr>
<tr>
<td></td>
<td>• Describe the function of the major enzymes involved in DNA replication.</td>
</tr>
<tr>
<td>Cell division and reproduction</td>
<td>Lecture topics</td>
</tr>
<tr>
<td></td>
<td>• Nature of genes and chromosomes.</td>
</tr>
<tr>
<td></td>
<td>• Cell division: mitosis and meiosis.</td>
</tr>
<tr>
<td>Learning outcomes</td>
<td>• To explain the difference between a gene and a chromosome.</td>
</tr>
<tr>
<td></td>
<td>• To describe the processes of mitosis and meiosis, the differences between them and their purpose.</td>
</tr>
<tr>
<td>Gene expression I: Transcription</td>
<td>Lecture topics</td>
</tr>
<tr>
<td></td>
<td>• DNA→RNA→protein.</td>
</tr>
<tr>
<td></td>
<td>• The genetic code.</td>
</tr>
<tr>
<td></td>
<td>• Transcription: The synthesis of RNA from a DNA template.</td>
</tr>
<tr>
<td></td>
<td>• Differences in gene expression between bacteria &amp; eukaryotes.</td>
</tr>
<tr>
<td>Learning outcomes</td>
<td>• Describe the genetic code.</td>
</tr>
<tr>
<td></td>
<td>• Explain how the instructions contained within DNA are transcribed into RNA.</td>
</tr>
<tr>
<td></td>
<td>• Define the three stages of transcription.</td>
</tr>
<tr>
<td></td>
<td>• State the main differences in gene expression between bacteria and eukaryotes.</td>
</tr>
<tr>
<td>Gene expression II: Translation</td>
<td>Lecture topics</td>
</tr>
<tr>
<td></td>
<td>• Overview: the translation of mRNA into amino acids.</td>
</tr>
<tr>
<td></td>
<td>• Transfer RNA and its role in translation.</td>
</tr>
<tr>
<td></td>
<td>• The ribosome as the protein synthesis factory.</td>
</tr>
<tr>
<td></td>
<td>• The three stages of translation.</td>
</tr>
<tr>
<td></td>
<td>• Control of gene expression.</td>
</tr>
<tr>
<td>Learning outcomes</td>
<td>• Explain the processes of transcription and translation and relate them to cell function and the role of ribosomes.</td>
</tr>
<tr>
<td></td>
<td>• Describe the basic structure and function of tRNA.</td>
</tr>
<tr>
<td></td>
<td>• Describe the three main stages of translation.</td>
</tr>
<tr>
<td></td>
<td>• Explain the main differences between control of gene expression in bacteria and eukaryotes.</td>
</tr>
</tbody>
</table>
| The polymerase chain reaction | **Lecture topics**  
• Polymerase chain reaction (PCR).  

**Learning outcomes**  
• To describe the PCR technique and the basic steps involved.  
• To list several applications of the PCR.  

| Mutation | **Lecture topics**  
• Errors in reproduction.  
• Environmental influences (radiation, chemicals, viruses).  
• “Beneficial mutations” and natural selection.  
• Loss of function or changed function.  
• Gene duplication.  
• Relationship to selected disease.  
• Mutations in the immune system.  

**Learning outcomes**  
• To explain at mechanisms by which mutations can arise.  
• Relate the occurrence of mutations to the outcomes for cells and whole organisms.  
• To appreciate the importance of the rate of mutation for the evolution of species.  

| Mendel's laws of heredity | **Lecture topics**  
• Mendel’s laws.  
• Essential concepts in genetics: allele vs. locus, genotype vs. phenotype, homozygosity vs. heterozygosity, recessive vs. dominant  

**Learning outcomes**  
• To describe Mendel's laws.  
• To explain the basis of inherited characteristics.  
• To explain why genotype does not always equal phenotype.  

| Mechanisms of inheritance | **Lecture topics**  
• Modes of inheritance (single-locus, Mendelian traits).  
• Inheritance of complex traits.  
• Vertical inheritance vs. horizontal gene transfer.  

**Learning outcomes**  
• To understand the varied modes of inheritance in different organisms.  

| Population genetics | **Lecture topics**  
• Hardy-Weinberg law.  
• Evolutionary forces that change allele and genotype proportions.  

**Learning outcomes**  
• To explain the evolutionary forces that influence population genetics using the Hardy-Weinberg model.  

| Course review |  
• Overview of BABS1201.  
• Exam structure and tips.  
• Student questions.  

---

Please note that the lecture topics and learning outcomes listed below are a guide only. Individual lecturers may provide you with updated topics and learning outcomes.
## Administrative Matters

### Expectations of Students

A pass in BABS1201 is conditional upon a satisfactory performance in both the assessment and practical programs. We expect that you will have:
- Attempted/submitted all assessment items.
- Attended all of the practical classes (an attendance record is kept).
- Kept an accurate and up-to-date laboratory manual, including the recording of all data and completion of calculations and questions.

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

### Assignment Submissions

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

### Equity and Diversity

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

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

### Student Complaint Procedure

<table>
<thead>
<tr>
<th>BABS School Contact</th>
<th>Science Faculty Contact</th>
<th>University Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marc Wilkins</td>
<td>Gavin Edwards</td>
<td>Student Conduct and Appeals Officer (SCAO)</td>
</tr>
<tr>
<td><a href="mailto:m.wilkins@unsw.edu.au">m.wilkins@unsw.edu.au</a></td>
<td><a href="mailto:g.edwards@unsw.edu.au">g.edwards@unsw.edu.au</a></td>
<td>within the Office of the Pro-Vice-Chancellor (Students) Registrar</td>
</tr>
<tr>
<td>Tel: 9385 3633</td>
<td>Tel: 9385 7111</td>
<td>Tel: 9385 8515</td>
</tr>
<tr>
<td></td>
<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 session or in the end of session exams, may have been affected by illness or other circumstances may apply for special consideration. For BABS1201, applications can be made for in-session assessments tasks and the final examination.  

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

Students should consult the “Special Consideration” section of Moodle for specific instructions related to each BABS course they are studying. Further general information on special consideration can also be found at [https://student.unsw.edu.au/special-consideration](https://student.unsw.edu.au/special-consideration). |
| --- | --- |
| How to apply for special consideration | Applications must be made via Online Services in myUNSW. **You must obtain and attach Third Party documentation before submitting the application.** Failure to do so will result in the application being rejected. Log into myUNSW and go to My Student Profile tab > My Student Services channel > Online Services > Special Consideration. After applying online, students must also verify their supporting documentation by submitting to UNSW Student Central:

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

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

Further details are also available:  
[https://my.unsw.edu.au/student/academiclife/ProfessionalAuthority.pdf](https://my.unsw.edu.au/student/academiclife/ProfessionalAuthority.pdf)  

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

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

In Session 1, the BABS1201 Supplementary Examination Period will be:  

**Saturday 14 July – Saturday 21 July**

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](http://www.lc.unsw.edu.au/plagiarism)

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

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

Individual assistance is available on request from The Learning Centre.

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

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

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

If you have not scored 100% in the quiz by 9am on the day of your practical class you will NOT be permitted to attend that lab class or any subsequent lab class until you have satisfied this requirement.
B. Session Tests 1 and 2 – 10% each

| Weeks 4 and 10 – online during your enrolled lecture time |

Each test covers both theory and practical material. Test one includes from Weeks 1 to 4 (inclusive) of session. Test two includes Weeks 5-9 (inclusive).

The format of the exam is multiple choice questions.

The exam will be online, during your enrolled lecture time. Students that are unable to access a device to complete either of these tests need to contact the course convenor at least one week prior so that a venue/device can be organised.

More information about this test will be provided in lectures and via Moodle.

If you miss this test due to illness or misadventure, please apply for special consideration online (see page 18 for instructions).
C. Science Communication Project

The Science Communication Project is a group assignment with multiple components that runs throughout the session. 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 their 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;

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 team PRESENTATION (10%) on your advanced biology topic for submission and showcasing at the end of session;

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;

8. Submit an individual REFLECTIVE SUMMARY (5%) that describes your personal learning from both positive and negative experience throughout the duration of the Project, including a peer evaluation of team member contributions to all components of the project, and how any problems could have been better managed by the team.

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.
<table>
<thead>
<tr>
<th>Assessable Component</th>
<th>Specific Learning Outcomes</th>
<th>Assessment Weighting</th>
<th>Due Date</th>
</tr>
</thead>
</table>
| **Scientific Literature Essay** | • Search for peer-reviewed scientific literature  
• Discriminate between primary and secondary research papers  
• Practice reflective writing and written communication skills | 10% | Practical 3 (Week 6/7) |
| **Presentation Pitch** | • Effectively communicate ideas and scientific concepts to peers  
• Provide constructive feedback to peers | 5% | Practical 4 (Week 8/9) |
| **Presentation** | • Effectively communicate ideas and scientific concepts to peers  
• Use peer feedback to improve/enhance content and/or delivery of presentation  
• Use creativity to develop and deliver an accurate and effective scientific presentation to peers | 10% | Practical 6 (Week 11/12) |
| **Portfolio** | • Thoroughly and reliably record/document team meetings/communications  
• Maintain an inventory of all work pertaining to design, development and delivery of scientific presentation | 5% | Practical 6 (Week 11/12) |
| **Reflective Summary** | • Maintain a diary of personal learning experiences gained throughout project  
• Employ reflective practices to write about personal responses to learning experiences, opinions, events and new information  
• Accurately document and fairly evaluate contributions of individual team members to the project  
• Define learnt skills | 5% | Practical 6 (Week 11/12) |

**Total Assessment Weighting:** 35%
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. The origin of blue eyes is a single human ancestor with a specific mutation in Herc gene [https://www.sciencedaily.com/releases/2008/01/080130170343.htm](https://www.sciencedaily.com/releases/2008/01/080130170343.htm)
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-has-the-most-foreign-dna-of-any-animal](https://sciencealert.com/the-tardigrade-genome-has-been-sequenced-and-it-has-the-most-foreign-dna-of-any-animal)
7. 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/](http://www.iflscience.com/health-and-medicine/this-biohacker-became-the-first-person-to-edit-his-own-dna/)
10. Do red heads have super powers? Does mutation in the MC1R gene also lead to increased tolerance to pain in red haired individuals?
14. A vitamin has been found that halts aging in muscle tissues and increases life span [http://www.iflscience.com/health-and-medicine/awesome-vitamin-found-halt-aging-muscle-tissues](http://www.iflscience.com/health-and-medicine/awesome-vitamin-found-halt-aging-muscle-tissues)
18. Humans are still evolving and we can watch it happen

19. Could this new patent yield a functional cure for Type I diabetes?

20. Which health supplements are not backed by science?

21. Scientists have found a woman whose eyes have a whole new type of colour receptor

22. Scientists think they finally know why our genes are made of DNA and not RNA

23. A brand new human organ has been classified

24. Forget what you learned in high school – this new carbon molecule has 6 bonds

25. Have scientists just announced our best shot at ending antibiotic resistance to date?

26. We might finally know what triggered living cells to evolve for the first time

27. An epigenetics gold rush: new controls for gene expression

28. An unexpected new lung function has been discovered and it could disrupt decades of scientific thought

29. Scientists have successfully reversed DNA aging in mice

30. Two drugs have been identified that could halt the progression of neurodegenerative diseases

31. The energy generators inside our cells reach a sizzling 50°C

32. Cell maps reveal fresh details on how the immune system fights cancer

33. Antibody-powered nucleic acid release using a DNA-based nanomachine

34. High intensity workouts could slow down your aging by almost a decade

35. First timeline of a cancer tracks tumours from origin to spread
tumours-from-origin-to-spread/?utm_campaign=Echobox&utm_medium=Social&utm_source=Facebook#link_time=1494933833

36. A diabetes drug may work by changing gut bacteria

37. A new study identifies 52 genes associated with human intelligence
https://www.facebook.com/nature/posts/10154856218303167

38. The brain starts to eat itself after chronic sleep deprivation

39. CRISPR kills HIV and eats Zika 'like Pac-man'. Its next target? Cancer
https://www.wired.co.uk/article/crispr-disease-ma-hiv

40. CRISPR gene editing can cause hundreds of unexpected mutations

41. This Is the first ever nanoscale image of a living cell membrane. It's beautiful.

42. Gastric bypass surgery gives patients a new set of helpful microbes

43. Revolutionary tattoos designed to change colour according to your health
http://www.sciencealert.com/researchers-design-tattoos-changes-colour-health

44. Is ADHD a sleep disorder? Stimulant drug improves symptoms
https://www.newscientist.com/article/mg23431283-100-is-adhd-a-sleep-disorder-stimulant-drug-improves-symptoms/?utm_campaign=Echobox&utm_medium=Social&utm_source=Facebook&cmpid=SOC%7CNSNS%7C2017-Echobox&utm_source=Facebook#link_time=1496396954

45. Can gene therapy be used to switch off asthma? http://www.sciencealert.com/gene-therapy-used-to-switch-off-asthma

46. Tea consumption leads to epigenetic changes in women

47. Designer viruses successfully stimulate the immune system to fight cancer

48. Lifelong protection from severe allergies could be possible with a new treatment

49. 100 year old drug produces temporary improved learning skills in autistic children

50. Scientists discover plants have 'brains' that determine when they grow

51. This cancer drug proves to be effective against multiple tumours

52. 5 kilograms of broccoli in a pill slashes diabetics' blood sugar
https://www.newscientist.com/article/2134735-5-kilograms-of-broccoli-in-a-pill-slashes-diabetics-blood-sugar/?cmpid=SOC%7CNSNS%7C2017-
53. A nine-year collaboration has just shown how sugar influences cancer cell growth
   http://www.sciencealert.com/a-nine-year-study-has-just-shown-how-sugar-exacerbates-cancer
54. New semi-synthetic organism can make molecules we’ve never seen before
55. Latest DNA analysis shows the Yeti are actually just a bunch of bears
56. Babies who get more cuddles have their genetics changed for years
   http://www.sciencealert.com/cuddling-babies-alters-their-genetics-dna-for-years
57. 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-alien-life
58. Poo pills really are becoming our answer to dangerous superbug infections
59. This protein in your brain could be at the heart of creating memories
60. World’s smallest tape recorder has been built inside a living bacterium
   http://www.sciencealert.com/smallest-tape-recorder-crispr-cas-bacterium
PROJECT TIMELINE

Check Moodle announcements at least weekly for updates and supporting materials to guide your project work.

Week 2/3 In Lab Class Practical 1:
- Form project groups
- Select project topic
- Schedule a meeting time and venue for your team in Week 3/4

Week 3/4 Individual (in own time):
- Team members should start individually researching their topic prior to their arranged meeting time in Week 3/4. Initial research could simply involve internet searches using your topic title and/or keywords within it. These searches may yield popular science communications, links to various multimedia communications (e.g. tv/radio excerpts, videos etc.), as well as papers from peer-reviewed scientific literature. All are acceptable results for your initial research. As you progress through the Project and related BABS1201 learning activities, you and your team members will become more confident about which resources/sources of information to use for the essay and presentation.
- Work through the ‘Scientific Literature’ lesson on Moodle. Other lessons on conducting a literature search and referencing may also be useful.

Week 3/4 Team Meeting (out of class):
- Scheduled meeting team meeting
- Confirm topic and team members
- 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.

Week 4/5 In Lab Class Practical 2:
- Consultation with tutor - Confirmation of Project team members and chosen topics.

Week 5/6 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 8/9 and final presentation is due in Week 12/13)

Week 5/6 Individual (in own time):
- Each team member should be individually researching and writing their “scientific literature essay”

Week 6/7 In Lab Class Practical 3:
- Consultation with tutor

Week 7/8 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 7/8 Individual (in own time):
• Each team member should continue with their individual scientific literature essay (due in next lab class, Practical 4).

**Week 8/9  In Lab Class Practical 4:**
• Project Presentation Pitches delivered in lab class. Your demonstrator will facilitate both the individual team pitches and the peer feedback sessions after each pitch
• Individual scientific literature essays due in this lab class. An electronic copy should be submitted online in Moodle and a hard copy should be given to your demonstrator at the beginning of class.

**Week 9/10  Team Meeting (out of class):**
• Scheduled meeting team meeting
• Continue with the plan for your team presentation

**Week 10/11  In Lab Class Practical 5:**
• Consultation with tutor

**Week 11/12  Team Meeting (out of class):**
• Finalise the plan of your presentation that details its format and indicates how individual presentation criteria (see details below) will be satisfied.
• Finalise your team’s portfolio for submission in Week 12/13

**Week 11/12  Individual:**
• Start compiling your personal Reflective Summary and Peer Evaluations (see below for guidelines) for submission

**Week 12/13  In Lab Class Practical 6:**
• Project Final Presentations. Submit your teams’ presentation onto Moodle as a single file.
• Each team will have a maximum of 5 minutes to showcase/highlight their presentation to the whole lab class. The teams will select one spokesperson for the showcase
• Your demonstrator will help facilitate the presentations for your group.
• Team Portfolios due.
• Individual Reflective Summary due. See below for guidelines.
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, as well as peer-reviewed scientific journal articles. Since scientific journal articles will be the most difficult to understand for non-experts, we recommend that you start by looking at alternative sources of information. Then, once you have a better 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 peer-reviewed literature used by the scientific community. One of the major objectives of this assignment is to ascertain whether YOU are able to differentiate between primary and secondary (review) journal articles, so your demonstrator or lecturers will NOT provide you with an answer if you ask them to check the articles you are considering. They will, however, discuss HOW to differentiate between the two in class.

**ESSAY INSTRUCTIONS**

Your essay task is to find a **REVIEW** article (i.e. 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. Remember that this is a **reflective** essay.
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 sub-discipline. 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, 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 screen for plagiarism, so it is particularly important.
D. Mastering biology – 5%

In weeks 3, 7, 8, 9 and 12 you will be provided access to an online quiz related to the concepts learned during the practical component of the course. Each quiz is worth 1% of your final assessment and should take you less than 30 minutes to complete (although it is not timed). The quiz will be accessible via Moodle on the week of the relevant practical and is due by the end of the following week (11:59pm on Friday of week 4, 8, 9, 10 and 13 respectively).

E. Final Theory Exam – 40%

The final examination is conducted externally during the UNSW June examination period. The exam can address ANY material covered in lectures and practical classes throughout the BABS1201 program. The format and weighting of questions in the final exam will be shown on the front cover of the exam paper, which will be posted on Moodle after submission to the exams branch part way through this session. PLEASE NOTE that the final exam is a COMPULSORY assessment and must be completed in order to satisfy the requirements for passing the course. If you miss this exam due to illness or misadventure, please apply for special consideration online (see page 18 for instructions).

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

CONTENTS
1. Health & safety guidelines
2. Online Laboratory Safety Quiz
3. Mastering Biology

OBJECTIVE
• Be aware of safe work practices in the laboratory.

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 everyone 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, non-absorbant 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:


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 12. If ANY of your answers were incorrect, then you MUST attempt the quiz again and keep repeating this process until you have scored a mark of 12/12 (that is, 100%).

Since it is crucial that you are aware of all important health and safety rules and regulations, you can attempt the quiz as many times as required for you to get ALL the answers correct.

**If you have not scored 100% in the quiz by 9am on the day of your practical class you will NOT be permitted to attend that lab class or any subsequent lab class until you have satisfied this requirement.**

3. **MASTERING BIOLOGY**

The purpose of the following exercises is to familiarize you with the system you will be using for the rest of your course. These exercises are not intended to teach or test your knowledge of any specific subject material. Therefore, you will not be penalized for using hints or submitting incorrect answers. Accessing Mastering Biology is via Moodle, and is demonstrated in your first lecture.

Items in Mastering Biology typically have an introduction, possibly figures or animations, and one or more parts for you to answer.

1. Navigate to the Mastering Biology via Moodle and complete the activity named “Introduction to Mastering Biology”.
2. If you have any trouble running the exercises you may need to adjust your browser settings, see here: [http://www.pearsonmylabandmastering.com/northamerica/masteringbiology/system-requirements/index.html](http://www.pearsonmylabandmastering.com/northamerica/masteringbiology/system-requirements/index.html)
3. Ensuring Mastering Biology works is your responsibility BEFORE your assessable quizzes commence. If you encounter problems, the course administrator or conveners will ask that you:
   - Have checked you have the correct system requirements
   - Have complete the “Introduction to Mastering Biology” module
   - Have accessed the Mastering Biology 24/7 help desk via Moodle
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
## Practical 1: Cell Structure 1

### BABS Teaching Laboratory

**Student Risk Assessment**

<table>
<thead>
<tr>
<th>Hazard</th>
<th>Risk</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological agent (Environmental samples – pond water)</td>
<td>Infection</td>
<td>Follow demonstrator’s instructions when handling biological agents.</td>
</tr>
<tr>
<td>Biological agent (Microorganisms, <em>Bacillus megaterium</em>)</td>
<td>Spills</td>
<td>PPE (lab coats, closed in shoes, goggles &amp; gloves as required).</td>
</tr>
<tr>
<td>Biological agent (Environmental samples – <em>Spirogyra</em>)</td>
<td>Cuts from broken glass</td>
<td>Adhere to aseptic techniques.</td>
</tr>
<tr>
<td>Razor blades, glass slides/cover slips</td>
<td></td>
<td>Notify demonstrator immediately of any spills or other incidents.</td>
</tr>
<tr>
<td>Electrical Equipment (microscope)</td>
<td>Electric shock/ electrocution Exposure to biological agents (see above)</td>
<td>Carry slides/cover slips in suitable containers and dispose of any broken/used items immediately in sharps containers provided.</td>
</tr>
<tr>
<td>Ergonomics</td>
<td>Postural damage from extended periods of time working at microscope or bench</td>
<td>Proper handwashing with antibacterial handwash before leaving the laboratory.</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.

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 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 the ‘Introduction to Lab safety’ section in the Course Manual.
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.
Check that you know how to access the Mastering Biology Quiz 1 ‘Prokaryotic versus Eukaryotic Cell Structure’ and know the due date. The quiz is worth 1% of your final assessment and should take you around 15 minutes to complete.

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.
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>Unable to focus on object</td>
<td>Cover-slip too thick</td>
<td>Replace</td>
</tr>
<tr>
<td></td>
<td>Slide up-side down</td>
<td>Invert slide</td>
</tr>
<tr>
<td></td>
<td>Focusing attempts too rapid</td>
<td>Use fine focus and adjust more slowly</td>
</tr>
<tr>
<td></td>
<td>Objective has insufficient resolving power</td>
<td>Use higher power</td>
</tr>
<tr>
<td></td>
<td>Objective covered with dried immersion oil from previous use</td>
<td>Clean with lens tissue and solvent</td>
</tr>
<tr>
<td>Specks in field of view</td>
<td>Dirt on eye lens of ocular</td>
<td>Clean with lens tissue</td>
</tr>
<tr>
<td></td>
<td>Dirt on condenser lens</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dirt on filter</td>
<td></td>
</tr>
<tr>
<td>Moving shadows in field</td>
<td>Air and/or water bubbles in immersion oil</td>
<td>Remove oil with lens tissue. Re-apply</td>
</tr>
<tr>
<td>Light suddenly reduced</td>
<td>No oil contact between oil immersion objective and slide</td>
<td>Adjust with course /fine focus control</td>
</tr>
</tbody>
</table>

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

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

Often it is sufficient just to know an approximate measurement of the size of the object you are viewing, i.e. whether it is about 10 \( \mu \)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 18 mm.

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

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

So, using the 10X objective:

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

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

Accurate measurements using the eyepiece scale

In order to provide an accurate scale for a drawing it may be necessary to have accurate measurements. A ‘ruler’ or micrometer is built into the eyepiece of your microscope. A microdot slide of the number ‘5’ has been provided as a trial slide. **PLEASE NOTE: make sure that your slide is positioned with the correct side facing upwards. If you cannot focus on the number ‘5’ at higher objectives, you may have the slide upside down!** With this slide on the stage and in focus, observe the eyepiece micrometer. Notice that the scale has 100 divisions.

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

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

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

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

Using the scale, how high and how wide is the ‘5’?

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

3. Look at your slide under low power.

   How many different types of organisms can you see?

   How many are unicellular and how many multicellular?

   Can you tell whether they are plants or animals? What characteristics might help you decide?

Some common protists:

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

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

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

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

**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.

![Figure 2: Paramecium 100x magnification](image)
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.
### Biological agent

*(Microorganisms, *Rhodospirillum* and *Anabaena)*

- **Razor blades, glass slides/coverslips**

<table>
<thead>
<tr>
<th>Hazard</th>
<th>Risk</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Infection</em></td>
<td>Spills</td>
<td>Follow demonstrators instructions when handling biological agents. PPCE (lab coats, closed in shoes &amp; 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. Proper handwashing with antibacterial handwash before leaving the laboratory.</td>
</tr>
<tr>
<td><em>Spills</em></td>
<td>Cuts from broken glass</td>
<td></td>
</tr>
<tr>
<td><em>Razor blade cuts</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Hazardous chemicals (nitroblue tetrazolium salt)

- **Eyes**: Causes irritation.
- **Skin**: Causes irritation.
- **Ingestion**: Harmful if swallowed.
- **Inhalation**: Causes respiratory tract irritation.

**Personal Protection**: Wear appropriate safety goggles, gloves and protective clothing.

**Eyes**: Immediately flush with plenty of water for at least 15 minutes.

**Skin**: Immediately flush area with soap and water.

**Label**: Hazard Warning: Caution! May cause eye and skin irritation. May cause respiratory and digestive tract irritation.

**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 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, Mastering Biology resources, 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.**

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

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

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

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

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

*Why do you think you can’t see such structures in the cells you stained?*
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. 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.
3. CELL WALLS, VACUOLES AND CHLOROPLASTS OF EUKARYOTIC CELLS

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>
<tbody>
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<td></td>
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</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% nigrosine 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 bacterial suspension on a slide, smear it as before, and allow it to dry under the lamp.

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

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

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

**What features can you see?**

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

**What shape are the bacteria?**

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

Figure 5:
5. **STAINING FIXED CELLS**

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

**Unfixed cells:**

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

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

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

   | Can you think of an explanation for this? |

**Fixed cells:**

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

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

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

   | What are the main features you see? |

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

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

Figure 6:

Write a brief explanation of the difference between the two slides.
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 focused.

The aims for this section are:

- Calculate and report on parameters involved in diffusion through a semi-permeable 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
### Physical injury (glass capillary tube)
- **Cuts.**
- Breaking glass 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 (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.**

### 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 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, Mastering Biology resources, 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 semi-permeable 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

**Work in pairs**

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

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

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

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

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

4. Examine the apparatus for leaks.

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

   Measure the level every 20 minutes for the next 2 hours and plot the results against time on the blank graph provided as Figure 2 (don't forget to give the figure a title).
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: \[ \Delta \pi = 1000 \, RT \, (C_i - C_o) \]

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

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

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

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

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

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

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

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

**Animal erythrocytes:**

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

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

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

**Table 1. Concentrations of 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>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.50</td>
<td>3.50</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.75</td>
<td>3.25</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.00</td>
<td>3.00</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.25</td>
<td>2.75</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2.50</td>
<td>2.50</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2.75</td>
<td>2.25</td>
<td>110</td>
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<tr>
<td>8</td>
<td>3.00</td>
<td>2.00</td>
<td>120</td>
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<tr>
<td>9</td>
<td>3.25</td>
<td>1.75</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>3.50</td>
<td>1.50</td>
<td>140</td>
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</table>
4. To each tube, add 0.1 ml sheep blood and shake well.

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

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

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

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

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

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

Solute permeability

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

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

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

11. Place 2 Ml of each of your solutions into separate small test tubes. Add 2 drops of blood to each tube and quickly stir.
12. Using a stop watch if necessary, note the time taken for complete lysis of the 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 2: Estimation of induced lysis of erythrocytes

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

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

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

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

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

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

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

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

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

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

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

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

<table>
<thead>
<tr>
<th>Tube</th>
<th>Volume of NaCl (mL)</th>
<th>Volume of distilled water (mL)</th>
<th>Final NaCl concentration (mM)</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>

Show your table to your demonstrator. While there is no one correct answer for the appropriate concentrations of NaCl to use, your tutor will review your calculations for the dilutions.

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

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

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

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

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

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

Table 4: Results

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

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

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

HOMEWORK: MASTERING BIOLOGY

Before your next laboratory class there are three Mastering Biology tutorial activities for you to complete. The activities will also help you prepare for your Mastering Biology Quiz 3 Photosynthesis, which is an assessable part of the course.

Work through the following three BioFlix tutorials:

<table>
<thead>
<tr>
<th>ITEM TYPE</th>
<th>TITLE</th>
<th>MEDIAN TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tutorial</td>
<td>Photosynthesis (1 of 3): Inputs, Outputs, and Chloroplast Structure (BioFlix tutorial)</td>
<td>14 min</td>
</tr>
<tr>
<td>Tutorial</td>
<td>Photosynthesis (2 of 3): The Light Reactions (BioFlix tutorial)</td>
<td>11 min</td>
</tr>
<tr>
<td>Tutorial</td>
<td>Photosynthesis (3 of 3): The Calvin Cycle (BioFlix tutorial)</td>
<td>10 min</td>
</tr>
</tbody>
</table>
### 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.

<table>
<thead>
<tr>
<th>Physical injury (plastic capillary tube)</th>
<th>Cuts.</th>
<th>Do not bend the capillary tube. <strong>Hold the tube close to the end being inserted</strong> through the bung and take care to apply force only along the axis of the capillary tube.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physical injury</strong></td>
<td>Breaking tube when inserting into rubber bung.</td>
<td></td>
</tr>
</tbody>
</table>

**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.

---

**Emergency Procedures**

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

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

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

Signature: _______________________________ Date: ______________________

Student number: _________________________
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.

Complete the following three Mastering Biology tutorial activities. The activities will also help you prepare for your Mastering Biology Quiz 3 Photosynthesis, which is an assessable part of the course.

<table>
<thead>
<tr>
<th>ITEM TYPE</th>
<th>TITLE</th>
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<tr>
<td>Tutorial</td>
<td>Photosynthesis (3 of 3): The Calvin Cycle (BioFlix tutorial)</td>
<td>10 min</td>
</tr>
</tbody>
</table>

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.](image)

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.](image)
Since the oxidising agent used by plants in intact chloroplasts is NADP+, the light reaction can be written as:

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

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

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

*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 in vitro (literally, in glass, that is a test-tube) reaction similar to the photosynthetic activity of the chloroplasts during in vivo (literally, in life) photosynthesis.

**Aim:**

The aim of this experiment is to demonstrate the transfer of electrons from chlorophyll a to a suitable electron acceptor and the subsequent replacement of those electrons from water molecules so releasing O₂.

**Preparing the chloroplasts:**

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

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

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

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

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

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

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

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

**Which reaction leads to this change in colour?**

**Effect of light intensity:**

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

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

**Table 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>
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<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>
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<tr>
<td>B. time for decolourisation (sec)</td>
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<tr>
<td>C. Rate of breakdown (A/B)</td>
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</table>
Express your results as a graph (Figure 3). Make sure your graph is fully labelled and accurately represents the data in the table.

What can you conclude from this experiment?

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

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

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

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

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

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

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

**Procedure:**

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.
<table>
<thead>
<tr>
<th>Question</th>
</tr>
</thead>
<tbody>
<tr>
<td>How reproducible are your results? How can you evaluate this?</td>
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<td></td>
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<tr>
<td>Why is it necessary to put soda lime into the respirometer?</td>
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<tr>
<td>What is the purpose of the sterilized mung beans? Can you think of an</td>
</tr>
<tr>
<td>alternative way of doing this?</td>
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<tr>
<td>Was there a difference in the oxygen consumption of the fresh and</td>
</tr>
<tr>
<td>sterilised mung beans? If so, can you explain this?</td>
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<tr>
<td></td>
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<tr>
<td>Did you observe water vapour within the respirometer? Where may this</td>
</tr>
<tr>
<td>water have come from?</td>
</tr>
</tbody>
</table>
Table 2.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Height of liquid (mm)</th>
<th>Volume of gas consumed (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>5</td>
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<td>45</td>
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</tbody>
</table>
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
## Practical 5: Genes 1: Mitosis and Cell Division

### Student Risk Assessment

<table>
<thead>
<tr>
<th>Hazards</th>
<th>Risks</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td>See below</td>
<td><strong>At all times</strong> follow demonstrators instructions when handling all biological substances, chemicals and equipment items. Notify demonstrator immediately of any spills or other incidents.</td>
</tr>
<tr>
<td>Physical injury (razor blades, needles)</td>
<td>Cuts</td>
<td>Do not walk around laboratory with exposed sharps. Dispose of all used blades in the sharps bins provided on your bench.</td>
</tr>
<tr>
<td>Hazardous chemicals (45% acetic acid mounting medium, 70% ethanol)</td>
<td>Do not get in on skin or clothing. If you do, wash the affected area for several minutes with running water.</td>
<td>Wear Personal protective equipment (gowns, gloves and enclosed shoes). Wash hands before leaving the laboratory. Wear safety glasses when working with concentrated acid. Wear gloves at all times.</td>
</tr>
</tbody>
</table>

### Emergency Procedures

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

### 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 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.

Complete the following three Mastering Biology tutorial activities. The activities will also help you prepare for your Mastering Biology Quiz 4, which is an assessable part of the course.

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

OBJECTIVES

- Describe human karyotyping and its role in ascertaining sex and detecting chromosomal abnormality.
- List and explain the 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. $G_1$ is the first growth phase, and $G_2$ 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 chromatin-staining dye (e.g. Giemsa). Finally, the cells are 'squashed' on microscope slides and cells with clearly visible chromosomes (generally at metaphase in the cell cycle) are identified under the microscope. Homologous chromosomes have distinctive lengths, centromere positions and staining patterns, and these features are used to identify and organise the chromosomes into a karyotype (see Figure 2). By convention, the karyotype is constructed by pairing the autosomes and arranging them in order of size (from largest to smallest) and centromere position (metacentric→submetacentric→acrocentric→telocentric; see Figures 2 and 3). Image analysis software is often used to build and analyse the karyotype.
1. **OBSERVING KARYOTYPES**

**Prepared slides**

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

**Abnormalities in a karyotype**

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

![Figure 2: Karyotype of a human male using Giemsa staining](image-url)
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).

![Diagram of meiosis process]

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.
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 pg of DNA which is too small a quantity to be seen on an agarose gel (of course it would be indistinguishable from the rest of the genome). If the 300 bp sequence can be selectively amplified a million-fold by PCR, then the 0.1 µg can be visualized on an agarose gel. This can be accomplished in an afternoon by the PCR technique.

**DNA sequence:**

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

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

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

2. Add 4 µl of PCR mix.

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

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

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

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

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

**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.

### Sharps Risks

**Physical injury**

Use caution working with pipette tips. Dispose of used tips in the sharps bins on your bench.

### Use of electrical equipment Risks

**Electrocution, fire, tripping over cables**

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.

### Chemicals (Taq DNA polymerase, Reaction buffer (10x), MgCl₂, dNTP’s, forward and reverse primers (oligonucleotides), template DNA (or cDNA), milliQ water) Risks

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.**

### UV light (Gel doc transilluminator) Risks

**UV damage to skin and/or eyes**

Ensure UV switches off when door is opened. If not, switch off equipment and immediately notify staff.

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<table>
<thead>
<tr>
<th>General</th>
</tr>
</thead>
<tbody>
<tr>
<td>See below</td>
</tr>
<tr>
<td><strong>At all times</strong> follow demonstrators instructions when handling all biological substances, chemicals and equipment items. Notify demonstrator immediately of any spills or other incidents.</td>
</tr>
</tbody>
</table>

### Personal Protective Equipment

- **Closed in Footwear**
- **Lab. Coat**
- **Gloves**

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

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

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

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

Student number:………………………………….
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.
Complete the following three Mastering Biology tutorial activity. This will also help you prepare for your Mastering Biology Quiz 5, which is an assessable part of the course.

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

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.

![Diagram of meiosis showing allele segregation](image)

**Figure 1:** Alleles segregate during meiosis.

*Why do the two chromatids of a chromosome have the same alleles (A and a) on one dyad?*
Using *Drosophila* to verify Mendel's first law of segregation:

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

**What size wings would the VV parent have?**

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

**What size wings would the vv parent have?**

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

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

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

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

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

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

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

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

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

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

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

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

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

<table>
<thead>
<tr>
<th></th>
<th>Full wings</th>
<th>Short wings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Your data</td>
<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.](image)

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?
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.

What is the genotype of all individuals in the $F_1$ generation?

What is the phenotype of all the individuals in the $F_1$ generation?

Which alleles are present in gametes produced by $F_1$ 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

Gametes from mother

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>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>full wing, red eye</td>
<td></td>
</tr>
<tr>
<td>full wing, white eye</td>
<td></td>
</tr>
<tr>
<td>short wing, red eye</td>
<td></td>
</tr>
<tr>
<td>short wing, white eye</td>
<td></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></th>
<th>Full wing + white eye</th>
<th>Full wing + red eye</th>
<th>Short wing + white eye</th>
<th>Short wing + red eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Your data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class total</td>
<td></td>
<td></td>
<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 7?

Table 1.

<table>
<thead>
<tr>
<th>MODE OF INHERITANCE</th>
<th>TRANSMISSION</th>
<th>PARENTS OF AFFECTED</th>
<th>SIBLINGS OF AFFECTED</th>
<th>OFFSPRING OF AFFECTED</th>
<th>POPULATION SEX RATIO OF AFFECTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autosomal Dominant</td>
<td>Never skips a generation</td>
<td>Aa x aa</td>
<td>1/2 affected</td>
<td>1/2 affected</td>
<td>1:1</td>
</tr>
<tr>
<td>X-linked Dominant</td>
<td>Never skips a generation</td>
<td>Affected father B- x hh or Affected mother b- x Bb</td>
<td>All sisters affected all brothers normal or 1/2 affected (both sexes)</td>
<td>Depends on sex of affected, as for previous generation</td>
<td>M:F</td>
</tr>
<tr>
<td>X-linked Recessive</td>
<td>Skips generations (through female carriers)</td>
<td>D- x Dd</td>
<td>All sisters: normal, 1/2 brothers affected</td>
<td>All normal</td>
<td>M&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 x 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></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number tested</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number with colour vision deficiency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence of colour vision deficiency (% total tested)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion of total colour vision deficiency individuals</td>
<td></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 100 volts) until the bromophenol blue marker dye has migrated half way.

10. While this is happening, perform the DNA precipitation (part 2 of this practical).

11. With the aid of your demonstrator, visualise the DNA in the gel using the Gel Documentation system.

**Analysis:**

Estimate the size of the bands on the agarose gel, using the molecular weight markers as a guide. The sizes of the marker bands are: 1000, 800, 600, 500, 400, 300, 200, 150, and 100 base pairs, with an additional faint band at 50 base pairs. With this information, you should be able to determine which individuals in the pedigree have a complete exon and which have a deletion. Use this to complete the following pedigree.

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?**