UNIVERSITI PERUBATAN ANTARABANGSA
INTERNATIONAL MEDICAL UNIVERSITY
MALAYSIA

MSc Molecular Medicine

MM301 Molecular Medicine

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Guidelines for References

This guide introduces you to the Vancouver referencing style, which uses a 'numericalendnote' approach.

1.0 WHAT IS REFERENCING?

It is a method used to demonstrate to your readers that you have conducted a thorough and appropriate literature search, and reading. Equally, referencing is an acknowledgement that you have used the ideas and written material belonging to other authors in your own work. As with all referencing styles, there are two parts: citing, and the reference list.

2.0 WHY SHOULD I REFERENCE?

Referencing is crucial to you to carry out successful research, and crucial to your readers so they can see how you did your research. Knowing why you need to reference means you will understand why it is important that you know how to reference.

1. Accurate referencing is a key component of good academic practice and enhances the presentation of your work: it shows that your writing is based on knowledge and informed by appropriate academic reading.
2. You will ensure that anyone reading your work can trace the sources you have used in the development of your work, and give you credit for your research efforts and quality.
3. If you do not acknowledge another person's work or ideas, you could be accused of plagiarism.

3.0 HOW DO I WRITE CITATIONS USING THE VANCOUVER STYLE?

Each piece of work which is cited in your text should have a unique number, assigned in the order of citation. If, in your text, you cite a piece of work more than once, the same citation number should be used. You can write the number in brackets or as superscript.

The Reference section must include all journal articles (both print and online), books and book chapters (both print and online), patents, theses and dissertations, and published conference proceedings, as well as in-press journal articles, book chapters, and books (publication title must be given) cited in the text. The Vancouver reference style is used, whereby the references are indicated by a number series in the text and listed in numerical order as they appear in the text under bibliography.

Only peer-reviewed articles and books may be used as reference materials in a laboratory report. Information from independent web-pages should be kept to a minimum. Proper citation is of paramount importance in a laboratory report as in any scientific papers.

3.1 Article from a journal:

The titles of journals should be abbreviated according to the style used in Index Medicus. Consult the list of Journals Indexed for MEDLINE, published annually as a separate publication by the National Library of Medicine.

Arendsen AF, Solimar MQ, Ragsdale RW. Nitrate-dependent regulation of acetate biosynthesis and nitrate respiration by *Clostridium thermoaceticum*. J Bacteriol. 1999; 181:1489-1495.

3.2 A chapter from a book:

Blaxter M. Social class and health inequalities. In: Carter C, Peel J, editors. Equalities and inequalities in health. London: Academic Press, 1976; p. 369-80.

3.3 Article from proceedings:

Harnden P, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ Cell Tumour Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer; 2002.

3.4 Online references:

1. Charlier, D., and N. Glansdorff. September 2004, posting date. Biosynthesis of arginine and polyamines. *In* R. Curtiss III et al. (ed.), EcoSal-*Escherichia coli* and *Salmonella*: cellular and molecular biology, chapter 3.6.1.10. [Online.] http://www.ecosal.org. ASM Press, Washington, D.C. *{For online-only books or continually updated Web resources [for the latter, posting or accession date is required, but publisher's name and location are optional].}*
2. Dimick, J. B., H. G. Welch, and J. D. Birkmeyer. 18 August 2004, posting {*or* revision} date. Surgical mortality as an indicator of hospital quality. JAMA 292. [Online.] http://jama.ama-assn.org/cgi/content/short/292/7/847. {*For online journals; page numbers may not be available.*}
3. Sullivan, C. J. (ed.). 1999-2001. Fungi: an evolving electronic resource for the microbiological community. ASM Press. [Online.] http://link.asmusa.de/link/service/books/91090. Accessed 7 September 2001. {*For online-only books.*}
4. Zellnitz, F., and P. M. Foley. 2 October 1998, posting {*or* revision} date. History of virology. Journal of Virology 1:30-50. [Online.] http://wwwavj.html. {*For online-only journals; page numbers may not be available.*}
5. Zheng, Z., and J. Zou. 5 September 2001. The initial step of the glycerolipid pathway: identification of glycerol-3-phosphate/dihydroxyacetone phosphate dual substrate acyltransferases in *Saccharomyces cerevisiae*. Journal of Biological Chemistry. doi:10.1074/jbc.M104749200. {*For papers published online in manuscript form.*}

Example:

In lymph node aspiration, mycobacteria were seen in routine May Grunwald Giemsa-stained smear as unstained rod-shaped structures in the background and within histocytes [1]. These are negative images of the bacilli [2, 3].

References:

[1] Jayaram G, Dashini M. Evaluation of fine needle aspiration cytology of salivary glands: an analysis of 141 cases. Malays J Pathol. 2001; **23(2)**: 93-100.

[2] Jannotta FS, Sidway MK. The recognition of mycobacterial infections by intraoperative cytology in patients with acquired immunodeficiency syndrome. Arch Pathol Lab Med. 1989; **113**: 1120-3.

[3] Maygarden SJ, Flanders EL. Mycobacteria can be seen as negative images in cytology smears from patients with immunodeficiency syndrome. Mod Pathol. 1989; **2**: 239-43.

Experiment 1: Estimation of protein concentration using the spectrophotometer
(Report writing: Full report)

1 Introduction

1.1 Spectrophotometer

The spectrophotometer is an optical instrument used in biological and chemical sciences. It is designed to transmit light of narrow wavelength ranges through a sample solution and, by comparison to the initial intensity of light reaching the solution, it indirectly measures the amount of light absorbed by the solutes in solution. Each solution with a different solute has its own characteristic absorption property or spectrum. The amount of light absorbed is directly proportional to the concentration of absorbing compounds in that sample, so a spectrophotometer can also be used to determine concentrations of compounds in solution. A solution, such as bromophenol blue, appears colored because it absorbs certain wavelengths of light in the visible spectrum and transmits or reflects others. However, some compounds absorb only wavelengths outside of the visible light spectrum, and that's why there are colorless solutions like water. Because different compounds absorb light at different wavelengths, a spectrophotometer can be used to distinguish compounds by analyzing the pattern of wavelengths absorbed by a given sample. Figure 1.1 shows the schematic diagram of the components in a spectrophotometer.

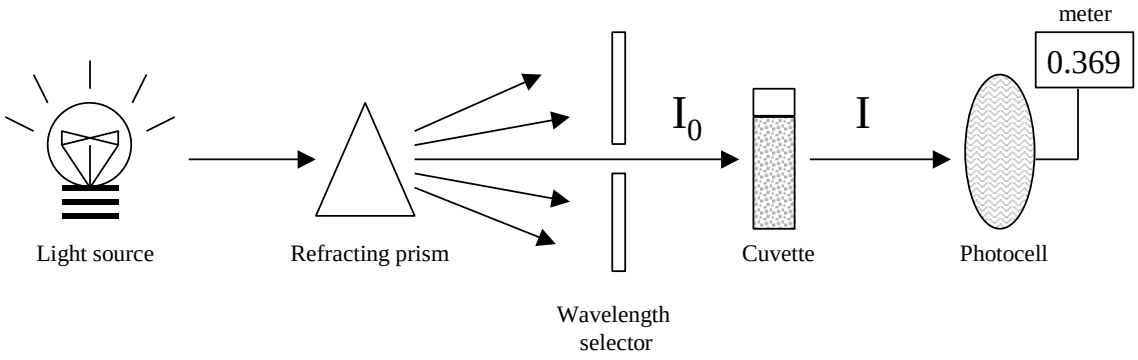

Figure 1.1: Schematic diagram of the components in a spectrophotometer. The arrows indicate light path from the light source.

The light from the light source of a spectrophotometer is separated into specific portions of spectrum by a prism or diffraction grating. A narrow portion of separated spectrum passes through a slit (the incident light, I_0) which is then passing through the sample solution in the cuvette. Light that passes through the sample solution (I) is detected by a photosensitive tube or photocell that measures the transmittance or absorbance value for the sample.

The amount of light absorbed by different solute in a sample solution can be measured in a number of ways:

(I)

The amount of light transmitted through a sample solution is referred to as transmittance (T). The formula of transmittance is

$$T = I/I_0$$

$$\%T = 100 \times T$$

I is the incident light, and I_0 is the energy of light transmitted through the solution (I). Absorbance (A) is the amount of light absorbed by a solution which is related logarithmically to transmission by the

$$A = \log_{10} I_0/I$$

At the end, $A = 2 - \log_{10} \%T$

(II)

According to Beer Lambert Law,

Absorbance, $A = \lambda bc$

the molar absorbtivity coefficient in L mol^{-1} cm^{-1}, a constant for a particular compound, different compound has different molar absorbtivity.
b is the path length (in cm), the path length of the cuvette in which the sample is contained
c is the concentration of the compound in solution, in mol L^{-1}

The Beer-Lambert law can be used to calculate the concentration of a solution if its molar absorbtivity coefficient is known. To determine the molar absorbtivity coefficient, we measure the absorbance of a known concentration of solution and then solve it by rearranging the equation as below:

$$\lambda = A/bc$$

No single method gives absolutely accurate results in all circumstances because different proteins have different composition of amino acids, which leads to the variable reactions when using different chromophores. A standard curve [absorbance (A) versus protein concentration] produced by measuring the absorbance of protein solutions of known concentrations (according to Beer-Lambert Law) is often used to estimate the protein concentration of an unknown solution.

1.2 Estimation of protein concentration

It is often important to know the concentration of protein in a biological material in biochemical analyses. Many methods of quantitative analysis of protein have evolved over the years to determine the concentration of protein in a solution. Most methods are based on the binding of a chromophore to specific amino acids or bonds in the protein. The resulting color development can be detected at some wavelength of visible light by spectrophotometer. No single method gives absolutely accurate results in all circumstances because different proteins have different composition of amino acids, which leads to the variable reactions when using different chromophores. A standard curve [absorbance (A) versus protein concentration] produced by measuring the absorbance of protein solutions of known concentrations (according to Beer-Lambert Law) is often used to estimate the protein concentration of an unknown solution.

This experiment is to acquaint you with the uses of the spectrophotometer and standard lab procedures for determining the amount of protein present in a solution by using method of Bradford protein assay. The Bradford protein assay is a colorimetric assay for determination of protein concentrations in solutions based on the change in absorbance in Coomassie Blue G-250 upon the binding of protein.

2 Objectives

2.1.1 To prepare a standard curve of absorbance versus protein concentration by using Bovine Serum Albumin (BSA).

2.1.2 To determine BSA concentration in two sample solutions.

2.1.3 To determine protein concentration in apple juice.

2.2 Materials

Bradford Reagent, Bovine Serum Albumin (BSA), apple juice sample, test tubes, cuvette, micropipette, tips.

Part 1: Preparation of standard curve of BSA

1. Prepare 6 test tubes and label them from 1 to 6.
2. Pipette the appropriate amount of distilled water and BSA into respective test tubes according to the Table 2.1.
3. Add 200 µl of Bradford Reagent into all the tubes.
4. Vortex briefly for proper mixing.
5. Leave the solutions for 2 min and measure the absorbance within 60 min at wavelength 595nm.
6. Plot standard curve of absorbance versus concentration of BSA.
7. Vortex briefly for proper mixing.
8. Leave the solutions for 2 min and measure the absorbance within 60 min at wavelength 595nm.
9. Calculate the average absorbance for tube 2-5.
10. Plot standard curve of absorbance versus concentration of BSA using Microsoft Excel.

Table 2.1 BSA standard preparation.

Tube	BSA stock,10 µg/mL (µL)	Distilled water (µL)	Bradford reagent (µL)	Concentration of standard (µg/mL)	Absorbance at 595 nm
1	0	800	200		
2	100	700	200		
3	200	600	200		
4	400	400	200		
5	600	200	200		
6	800	0	200		

Part 2: Determination of BSA concentration in two sample solutions

1. You are given two sample solutions in tubes A and B with unknown BSA concentration.
2. Pipette the samples A and B into two test tubes separately.
3. Add distilled water and Bradford Reagent according to the amount shown in Table 2.2.
4. Vortex and leave for 2 min.
5. Measure the absorbance at wavelength 595nm.
6. Determine the BSA concentration of samples A and B from standard curve drawn in Part 1.

Table 2.1 Unknown BSA concentration.

Tube	BSA unknown concentration (µL)	Distilled water (µL)	Bradford reagent (µL)	Absorbance at 595 nm
A	80	720	200	
B	80	720	200	

Part 3: Determination of protein concentration of an apple juice

1. You are given a sample solution of apple juice in tube C.
2. Pipette the sample C, distilled water and Bradford Reagent into a test tube according to the amount shown in Table 2.3.
3. Vortex and leave for 2 min.
4. Measure the absorbance at wavelength 595nm.
5. Determine the protein concentration of sample C by using the standard curve from Part 1.
6. Compare your result with the protein concentration stated in the nutritional information of the apple juice shown in Figure 1.2. Discuss what the possible reason for the discrepancy are. Is the Bradford method suitable for the analysis of the protein content in food? Why? Include the answers in your report under Discussion.

Include the answers to the questions below in your report under Discussion:

1. State two advantages of using Bradford protein assay compare to other methods?
2. Give one disadvantage of Bradford method in measuring protein concentration.

Table 2.3

Sample	Sample solution (μl)	Distilled water (μl)	Bradford reagent (μl)	Absorbance at 595nm
Apple juice	80	720	200	

Figure 1.2: Nutritional value of apple juice

Risk assessment

Experiment 2: Estimation of protein concentration

Compound	**Hazard**	**Handling (besides wearing gloves and goggles)**	**Spillage**	**Disposal**
Bradford Reagent	Corrosive. Causes burns. Harmful by inhalation, in contact with skin and if swallowed. Harmful: possible risk of irreversible effects through inhalation, in contact with skin, and if swallowed.	Do not breathe vapor. Do not get in eyes, on skin, on clothing. Avoid prolonged or repeated exposure. Keep tightly closed. Store at 2-8°C.	Cover with dry lime or soda ash, pick up, keep in a closed container, and hold for waste disposal. Ventilate area and wash spill site after material pickup is complete.	Closed waste container

Wear gloves and goggles at all time!

Safety information can be found in MSDS (sources of MSDS: Laboratory office and website such as www.fisher.com.my, www.merck.com)

Experiment 2: Using microscope to study cells
(Report writing: Simple report)

Objectives

1. To identify and know the functions of the different parts of the microscope
2. To learn how to use microscope
3. To prepare a wet mount
4. To identify the organelles of plant and animal cells
5. To learn how to determine the magnification of the specimen

Materials

Onion bud leaves, acetocarmine stain, methylene blue, immersion oil, microscope, glass slides, cotton bud stick, cover slips, blade, prepared microscope slides.

Procedure

Part 1: Main parts of the light microscope

A light microscope has the following basic systems:

Illumination	Shed light on the specimen
Light source	Produces light. Controlled by on/off switch
Light intensity knob	Alters the brightness of light
Condenser	Focuses light from the light source onto the specimen
Iris diaphragm	Controls the amount of light entering and leaving the condenser
Specimen control	Hold and manipulate the specimen
Stage	Where the specimen rests.
Clips	Hold the specimen still on the stage
Micromanipulator	Used to move the specimen horizontally along the x and y axes
Lenses	
Objective lens	Gathers light from the specimen and is an initial magnifier
Eyepiece lens	Second magnifier. Transmits and magnifies the image from the objective lens
Microscope tube	Supports the eyepiece at the proper distance from the objective lens
Nosepiece	Rotating mount that holds objective lenses
Focus	Position the objective lens at the proper distance from the specimen
Coarse-focus knob	Used to bring the specimen into the focal plane of the objective lens
Fine-focus knob	Used to make fine adjustments to focus the image
Support	
Arm	Curved portion that holds all the microscope parts at a fixed distance
Base	Supports the weight of all of the microscope parts

Label the diagram of light microscope in Figure 2.1 according to the parts listed.

Some of the parts mentioned above vary between microscopes. Microscopes come in two basic configurations: upright and inverted. The microscope shown in the diagram is an upright microscope, which has the illumination system below the stage and the lens system above the stage. An inverted microscope has the illumination system above the stage and the lens system below the stage.

Figure 2.1: Light Microscope

Part 2: How to use microscope

1. When you carry the microscope, support the arm with one hand, and support the bottom with the other hand.
2. Plug in the power supply of the microscope and switch on the light source.
3. Place a specimen slide on the stage. Make sure the slide is not placed upside down. Secure the slide by using the clips of the mechanical stage.
4. Rotate the nosepiece to the 10X objective, and raise the stage to its maximum.
5. With the light intensity knob, decrease the light while using the low magnification objective.
6. Move the stage with the coarse-focus knob to bring the desired section of the slide into the field of view.
7. Focus the specimen by using the coarse and fine focusing knobs.
8. Increase the magnification power by changing to 40X objective.
9. Increase the light by turning the light intensity knob until a bright but comfortable illumination is achieved.
10. Focus the specimen by turning the fine focusing knob.
11. When change to 100X objective, turn the 40X objective away from the slide, add one drop of immersion oil on the specimen slide before turning to 100X objective.
12. Focus the specimen by using ONLY the fine focusing knob. BEWARE! DO NOT adjust the focusing knob too much until you crash the slide with the 100X objective.
13. When the observation has been recorded, rotate the nosepiece to its original position (10X objective) and remove the slide from the stage.
14. If immersion oil was used, wipe it from the objective with lens paper at the end of each session.
15. Turn off the lamp, unplug, and put on the cover.

CAUTION: Only the 100X objective can be used for viewing under immersion oil. All other lenses are to be used without immersion oil; keep them dry and avoid applying oil or other liquids to these lenses.

Part 3: How to determine the magnification of the specimen

Magnification is calculated by multiplying the magnification of the objective lens with the magnification of the eyepiece. Magnification tells us how many times the specimen we observe on the slide is being magnified. The magnification of microscope lenses is engraved on the lens casing.

For example:
If you use 10X eyepiece with a 40X objective lens,

10 x 40 = 400
Magnification = 400X

Part 4: How to prepare a wet mount

1. You are given a segment of onion bulb leaf.
2. Peel the onion epidermis from the inner side of the onion bulb leaf with a blade.
3. Place the onion epidermis on a microscope slide.
4. Add a drop of water on the slide. At times, you may add the water on the slide before placing the specimen. The size of the drop should not be so large that the cover slip floats and it should not be so small that it doesn't fill the entire space under the cover slip.
5. Place the cover slip slowly on top of the specimen by first, holding its sides and lay its bottom edge on the slide close to the specimen at about 45^{o}.
6. Then, slowly lower the cover slip. Avoid entrapment of air bubbles. If you get air bubbles, gently press on the cover slip to move them to the edge. If there are dry areas under the cover slip, add more water at the edge of the cover slip. The excess water can be absorbed with paper towel.
7. Observe the specimen under the microscope start with the lower power (4X and 10X) to the higher power (40X).
8. Draw a schematic diagram of 4 to 5 onion epidermis cells at 10X and 40X objectives separately. Label your drawings and state the magnification of each drawing. ONLY labels organelles that you observe under light microscope.

STANDARD RULES: In each of your drawing of microscopic specimen, you should include labeling, magnification and title of your drawing (the title should be at the bottom of your drawing).

Part 6: How to stain your specimen

1. Prepare a new slide with a new piece of onion epidermis.
2. Repeat steps 2 to 6 of Part 4, but now replace the water with a drop of acetocarmine stain onto the specimen.
3. Observe the specimen under the microscope start with the lower power to the higher power.
4. Draw a schematic diagram of 4 to 5 onion epidermis cells at 10X and 40X objectives separately. Label your drawings and state the magnification of each drawing. State the differences of the stained and unstained (from Part 4) onion epidermis slides observed under light microscope. ONLY labels organelles that you observe under light microscope.

Part 7: Human cheek cells

1. Place a drop of methylene blue on a microscope slide.
2. Using a toothpick, gently scrape the inside of you cheek which will collect some cheek cells.
3. Place the toothpick into the methylene blue and mix.
4. Cover the microscope slide with cover slip.
5. Remove any excess liquid around the cover slip using a blotting paper.
6. Find and observe the cheek cells under low power (10X) and high power (40X and 100X).
7. Draw a schematic diagram of 2 to 3 cheek cells under high power 100X. Label your drawings and state the magnification of the cheek cells.

2.3 Part 8: Examine human red blood cells

1. You are given a prepared microscopic slide of human red blood cells.
2. Observe the slide under light microscope.
3. Draw 2 to 3 red blood cells under high power 100X. Label your drawing and state the magnification.
4. Describe the differences between the human red blood cells and cheek cells observed under light microscope.

Part 9: Bacterial cell under light microscope

1. You are given a prepared microscopic slide of *Bacillus cereus*.
2. Observe the slide under light microscope.
3. Draw 2 to 3 bacteria cells under high power 100X. Label your drawing and state the magnification.

Include the answers to the questions below in your report under Discussion:

1. State the differences between plant and animal cells from your observation under the light microscope in this practical.
2. State the differences between prokaryotic and eukaryotic cells from your observation under the light microscope in this practical.
3. What is the purpose of staining the specimen?
4. List the organelles of animal and plant cells that you observe under the light microscope in this practical. What is the limitation of light microscope?

Risk assessment

Experiment 3: Using microscope to study cells

Compound	**Hazard**	**Handling (besides wearing gloves and goggles)**	**Spillage**	**Disposal**
Acetocarmine stain	Causes severe irritation and burns. Harmful if swallowed. Avoid breathing vapor or dust.	Use with adequate ventilation. Avoid contact with eyes (use goggles), skin, and clothes.	Absorb spill with inert material, then place in a chemical waste container. Neutralize with a weak base.	Closed waste container
methylene blue	Harmful if swallowed. May cause irritation. Avoid breathing vapors, or dusts. Use with adequate ventilation. Avoid contact with eyes, skin, and clothes.	Keep in a tightly closed container, stored in a cool, dry, ventilated area. Protect against physical damage. Avoid dust formation and control ignition sources.	Sweep up and place in suitable (fiberboard) containers for reclamation or later disposal	Closed waste container

Wear gloves and goggles at all time!

Safety information can be found in MSDS (sources of MSDS: Laboratory office and website such as www.fisher.com.my, www.merck.com)

Experiment 3: Mitosis & Meiosis
(Report writing: full report)

Objectives

1. To identify the different stages of mitosis in the prepared slides of whitefish blastula and *Allium cepa* (onion root tip).
2. To identify the different stages of meiosis in *Valanga nigricornis* (grasshopper testis) and *Gloriosa superba* (lily anther).
3. To identify the differences between meiosis and mitosis stages.

Materials

Prepared microscope slides: Whitefish blastula, *Allium cepa* (onion root tip), *Gloriosa superba* (lily anther), *Valanga nigricornis* (grasshopper testis).

Procedure

MITOSIS

Part 1: Identify the different stages of mitosis in* whitefish blastula and *Allium cepa.

1. You are given a prepared microscopic slide of whitefish blastula and *Allium cepa* (showing mitosis in onion root tips).
2. Observe the slide under light microscope.
3. Find out the different stages of mitosis.
4. Draw one cell of each stage of mitosis: interphase, prophase, anaphase, metaphase and telophase.
5. Describe the differences between the mitosis in animal cell and plant cell.

MEIOSIS

***Part 2: Identification of different stages of meiosis in grasshopper and Gloriosa superba* (lily anther)**

1. You are given two prepared slides of *Valanga nigricornis* (showing grasshopper spermatogenesis) and lily anther (showing different stages of meiosis).
2. Observe the slides under light microscope.
3. Find out the different stages of meiosis in both slides.
4. Draw one cell of each meiosis stage you find in both slides.
5. Describe what happens in each of the stages.

Part 3: The differences between meiosis and mitosis stages

1. You are given photographs of mitosis and meiosis stages in lily.
2. Compare the mitosis and meiosis stages on the photographs.
3. Find out the differences between meiosis and mitosis stages in lily.
4. Describe the differences in your report under Results.

Include the answers to the questions below in your report:

1. State the definition of bivalent, and homologous chromosomes in your report under Discussion.
2. Briefly explain (in one page) the process of spermatogenesis in animal and sporogenesis in plant in your report under Introduction.

Risk assessment

Experiment 3: Mitosis and Meiosis

N.A.

Experiment 4: Patterns of Inheritance (ELAB)
(Report writing: simple report)

Introduction

FlyLab will allow you to play the role of a research geneticist. You will use BiologyLabs FlyLab online software to study important introductory principles of genetics by developing hypotheses and designing and conducting matings between fruit flies with different mutations that you have selected. Once you have examined the results of a simulated cross, you can perform a statistical test of your data by Chi-square analysis and apply these statistics to accept or reject your hypothesis for the predicted phenotypic ratio of offspring for each cross. With FlyLab, it is possible to study multiple generations of offspring, and perform testcrosses and backcrosses. FlyLab is a very versatile program; it can be used to learn genetic principles such as dominance, recessiveness, and Mendelian ratios, or more complex concepts such as sex-linkage, epistasis, recombination, and genetic mapping.

Objectives

The purpose of this laboratory is to:

1. simulate basic principles of genetic inheritance based on Mendelian genetics by designing and performing crosses between fruit flies
2. help you understand the relationship between an organism's genotype and its phenotype
3. demonstrate the importance of statistical analysis to accept or reject a hypothesis.

Part 1: Performing Monohybrid and Dihybrid Crosses

(i) Monohybrid

1. To begin a cross, you must first select the phenotypes of the flies that you want to mate. Follow the directions below to create a monohybrid cross between a wild-type female fly and a male with sepia eyes.

2. To design a wild-type female fly, click on the Design button below the gray image of the female fly. Click on the button for the Eye color trait on the left side of the Design view. The small button next to the words "Wild Type" should already be selected (bolded). To choose this phenotype, click the Select button below the image of the fly at the bottom of the design screen. Remember that this fly represents true-breeding parent that is homozygous for wild type alleles. The selected female fly now appears on the screen with a "+" symbol indicating the wild-type phenotype.

3. To design a male fly with sepia eyes, click on the Design button below the gray image of the male fly. Click on the button for the Eye Color trait on the left side of the Design view. Click on the small button next to the word "Sepia." The male fly now appears on the screen with the abbreviation "SE" indicating the sepia eye mutation. This fly is homozygous for the sepia eye allele. These two flies represent the parental generation (P generation) for your cross.

4. To select the number of offspring to create by this mating, click on the popup menu on the left side of the screen and select 10,000 flies.

5. To mate the two flies, click on the Mate button between the two flies. Note the fly images that appear in the box at the bottom of the screen. Scroll up to see the parent flies and down to see the wild type offspring. These offspring are the F_1 generation. Note: The actual number of F_1 offspring created by FlyLab does not exactly equal the 10,000 offspring that you selected. This difference represents the experimental error introduced by FlyLab.

6. Predict the phenotypic ratio that you would expect to see for the F1 offspring of this cross and describe the phenotype of each fly in your results.

7. Click on the Analyze Results button on the lower side of the screen. A panel will appear with a summary of the results for this cross. Note the number of offspring, proportion of each phenotype and observed ratios for each observed phenotype. Click the Add Data to Notebook button at the top panel. Copy the results and label the result as "F1 generation for monohybrid cross".

8. Click the Close button to close this panel and return to the Mate screen by clicking Return to Lab button.

9. Next is to set up a cross between two F_1 offspring to produce an F_2 generation. Click the Select button below the female wild-type fly image, then click the Select button below the male wild-type fly image and click Mate button. The F_2 generation of flies now appears in the box at the bottom of the screen.

10. Copy the results and label the result as "F1 generation for monohybrid cross".

11. Click on the Analyze Results button and click the Add to Notebook button. Copy the results and label the result as "F2 generation for monohybrid cross".

12. Next, perform a Chi-square analysis to test your hypothesis. Click on the Chi-Square Test button on the lower left side of the screen. To ignore the effects of sex on this cross, click on the Ignore Sex button.

13. Enter your predicted ratio in the box under the Hypothesis column.

14. Click the Test Hypothesis button at the bottom of the panel. Note the level of significance displayed with a recommendation to either reject or not reject your hypothesis. What was the recommendation from the Chi-square test? Was your ratio accepted or rejected?

15. Click the Add to Notebook button to copy the results of this test to your lab notes.

(ii) Dihybrid

16. Design a dihybrid cross by selecting and crossing an ebony body female fly with a male fly that has the vestigial mutations for wing size. Analyze the results of each cross by Chi-square analysis and save your data to your lab notes as previously described in the assignments for a monohybrid cross.

Part 2: Testcross

*A **testcross** is a valuable way to use a genetic cross to determine the genotype of an organism that shows a dominant phenotype but unknown genotype. For instance, using Mendel's peas, a pea plant with purple flowers as the dominant phenotype could have either a homozygous or a heterozygous genotype. With a testcross, the organism with an unknown genotype for a dominant phenotype is crossed with an organism that is homozygous recessive for the same trait. In the animal- and plant-breeding industries, testcrosses are one way in which the unknown genotype of an organism with a dominant trait can be determined. Perform the following experiment to help you understand how a testcross can be used to determine the genotype of an organism.*

1. Design a female fly with brown eye (BW) color (keep all other traits as wild-type), and design a male fly with ebony body color (E; keep all other traits as wild-type). Mate the two flies. Examine the F_1 offspring from this cross and save your data to your lab notes. Add to your data any comments that you would like.

2. To determine the genotype of an F_1 wild-type female fly, design a male fly with brown eye color and ebony body color, then cross this fly with an F_1 wild-type female fly. Examine the results of this cross and save the results to your lab notes.

   Discuss the following:

   a. What was the phenotypic ratio for the offspring resulting from this testcross?

   b. Based on this phenotypic ratio, determine whether the F_1 wild-type female was double homozygous or double heterozygous for the eye color and body color alleles.

   c. Explain your answer. If your answer was double homozygous, describe an expected phenotypic ratio for the offspring produced from a testcross with a double heterozygous fly. If your answer was double heterozygous, describe an expected phenotypic ratio for the offspring produced from a testcross with a homozygous fly.

Part 3: Sex Linkage

1. Cross a female fly with a tan body with a wild-type male. Record the phenotypes and ratios did you observe in the F1 generation.

2. Next, mate two F1 flies and observe the results of the F2 generation.

   Discuss the following:

   a. Based on what you know about Mendelian genetics, did the F2 generation demonstrate the phenotypic ration that you expected? If not, what phenotypic ratio was obtained with this cross?
   b. Explain your answer.

3. Perform a new cross by crossing a female fly with the vestigial wing size mutation and a white-eyed male. Describe the phenotypes obtained in the F2 generation. Examine the phenotypes and sexes of each fly.

   Discuss the following:

   a. Explain the ratio you obtained by using Law of probability.

   b. Is there a sex and phenotype combination that is absent or underrepresented? If so, which one?

   c. What does this result tell you about the sex chromosome location of the white eye allele?