Country: _____

Student Code: _____

23rd INTERNATIONAL BIOLOGY OLYMPIAD

8th – 15th July, 2012

SINGAPORE

PRACTICAL TEST 1

CELL & MOLECULAR BIOLOGY

Total points: **100**

Duration: **90 minutes**

Dear Participants

- In this test, you have been given the following task:

  Task: Gene mapping by restriction endonuclease digestion of DNA fragments

  Part A. Confirmation of insertion of human DNA in a cloning plasmid. (80 points)

  Part B. Determination of orientation by which the fragment is inserted. (20 points)

- Use the **Answer Sheet**, which is provided separately, to answer all the questions.
- The answers written in the Question Paper will **NOT** be evaluated.
- Write your answers legibly in ink.
- Please make sure that you have received all the materials and equipment listed for each task.

  If any of these items are missing, please raise your hand **immediately**.
- Stop answering and put down your pen IMMEDIATELY when the bell rings.
- At the end of the test, place the Answer Sheet and Question paper in the envelope provided.

  Our Assistants will collect the envelope from you.

Have fun and Good Luck! ☺

Materials and equipment:

Materials and equipment	**Quantity**	**Unit**
restriction endonuclease RE1 (*Nde*I) (kept on ice)	4 μl	tube
restriction endonuclease RE2 (*Eco*RI) (kept on ice)	4 μl	tube
DNA test samples in enzyme buffer (labelled T) (on ice)	10 μl x 4	tube
miliQ water (labelled W)	1	tube
DNA electrophoresis gel tank and power supply	1	set
micropipettes and tips in boxes (p10, p100)	2	piece
stopwatch	1	piece
DNA ladder (as internal size markers, L1 for 100 bp range and L2 for 1 kbp range) (on ice)	2	tube
DNA loading dye (blue in colour)	1	tube
pre-cast gel in holder (already placed in running buffer)	1	piece
large petri dish (for placing the gel for imaging purposes)	1	piece
card with your country code (in a clip holder): for signalling for assistance	1	piece
floating rack (labelled with your country code)	1	piece
micro-centrifuge	1	set
water-bath 37 ºC (there is one assigned for your usage)	1	set
gel doc (there is one assigned for your usage)	1	set

Task (100 points)

Gene mapping by restriction endonuclease digestion of DNA fragments

Introduction

Genetic mapping is routinely used in analysing the order and the identities of DNA fragments. This technique is based on the unique profiles of DNA fragments generated after DNA digests with specific combination of restriction endonucleases (RE) and revealed by DNA gel electrophoresis. It is extremely powerful for gene cloning, studying gene function and regulation, for finding candidate genes for diseases and their diagnosis and also as a forensic tool.

Part A. Confirmation of insertion of human DNA in a cloning plasmid. (**80 points**)

Using this technique, you are now tasked to confirm that a fragment of human DNA "X" (approximate size: 760 base pairs) has been inserted into a cloning plasmid or vector "V" (circular and approximate size: 2570 base pairs). You are required to design and carry out DNA digests by incubating DNA "T" with the restriction endonucleases by following the general protocol of incubation and electrophoresis given (details described below). After the gel electrophoresis, your results will be revealed by DNA staining (this will be performed by lab technicians), analysed and data interpreted.

Protocol and Procedures

1. Design your DNA digests (you may do a maximum of 4 tubes) in a total volume of 20 µl by using the Table in **the Answer Sheet**.

   Q1.1 **(20 points)** Record the desired amounts of reagents in your plan. One example is already given for Tube 2 in the table provided. All units are in µl.

2. Prepare the mixtures by carefully pipetting the correct amount of the reagents and gently mix them by pipetting them up and down in each tube. Label the tubes. Do not contaminate one sample with another when preparing the mixture. Use a clean pipette tip for each operation. Note: use p10 micropipette (white-coded) for pipetting reagents of less than 10 μl. [NOTE: there will be a penalty of 20 points if additional samples are requested. Please prepare the samples carefully.]

3. Spin down the mixture by placing all four tubes in the micro-centrifuge (please balance the spin by placing tubes opposite to each other). During preparation and after spinning, always keep the tubes on ice.

4. After all the tubes have been prepared, remove them from the ice and place them into the labelled floatation rack and incubate them for 20 minutes (stopwatch is provided) at 37 ºC in the water bath assigned to you. Make sure that you retrieve your own samples after the 20 minutes incubation time.

5. During this 20 minute incubation duration, answer the following questions **in the Answer Sheet**:

Q1.2 **(2 points × 5 = 10 points)** Indicate true statement(s) with a tick (✓) and false statement(s) with a cross (✗).

a. Each RE cuts DNA at a specific sequence.

b. Each RE cuts DNA only at the 3' and 5'ends.

c. RE are most effective in digesting DNA at 4 ºC.

d. RE can be kept at room temperature for months.

e. Unlike exonucleases, RE only cuts DNA internally.

Q1.3 **(2 points × 5 = 10 points)** Which of the following principles is true of separating DNA by gel electrophoresis? Indicate true statement(s) with a tick (✓) and false statement(s) with a cross (✗).

a. DNA fragments are overall positively charged.

b. The smaller DNA fragments move faster across the gel under the electric current.

c. The smaller DNA fragments are lesser charged than the larger fragments hence they move faster across the gel.

d. The relative density of the gel matrix affects how long the separation takes.

e. The voltage applied to the electrophoresis is determined by how much DNA is loaded in the gel.

6. When the 20 minutes of DNA digests duration is up, retrieve your own tubes from the water bath.

7. Add 4 μl of DNA loading-dye (blue colour). Mix them well by pipetting the mixture up and down and spin down any residual liquid using the micro-centrifuge.

8. Using the p100 micropipette (yellow-coded), load 15 μl of the sample mixture of DNA digests with the loading dye into the "wells" of the agarose gel provided. Make sure that you position the pipette tips carefully on top of the wells and gently deliver the mixture to the wells without spilling them. Load 15 μl of each of the Markers, L1 and L2. Add your samples according to the following scheme of lanes, starting from the left end of the gel.

Marker L1	Tube 1	Tube 2	Tube 3	Tube 4	Marker L2

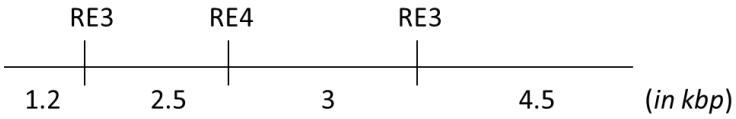
9. Cover the gel with a lid and connect the power supply to run at 100 volts for 20 min. Please be careful and do not touch any part of the electrodes and power supply.

10. Check regularly that the samples have entered the wells and are indeed running towards the positive electrode. If you need help from the technicians to ensure proper runs for the samples, please signal for assistance by clipping your signal card at the edge of right wall of your cubicle.

11. While waiting for the gel run, answer the following questions **in the Answer Sheet:**

Q1.4 **(20 points)** Consider the following scenario: A piece of linear human DNA (1 kbp) was digested by a particular enzyme RE3, resulting in 2 fragments of 650 bp and 350 bp. The same piece of 1 kbp DNA was digested with another enzyme RE4, releasing 2 fragments of 800 bp and 200 bp. And when this 1 kbp DNA was digested with RE3 and RE4 together, 3 fragments of DNA were generated, 650 bp, 200 bp and 150 bp.

Sketch a linear map of this piece of DNA by indicating the position of RE3 and RE4 digests in the space provided. An example of such a sketch is provided below as a guide.



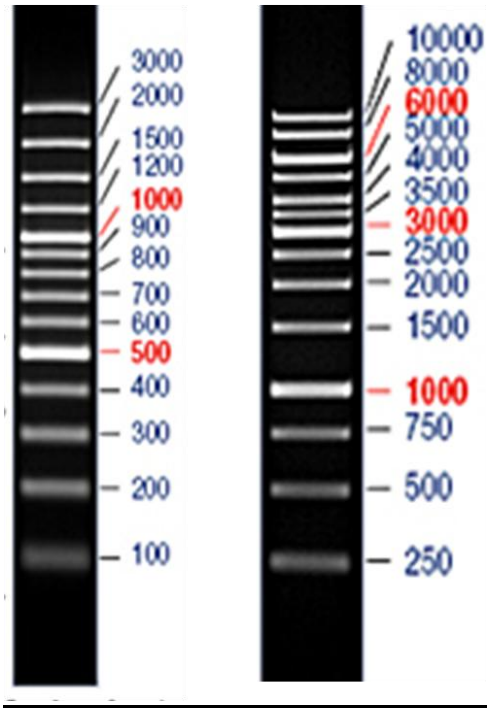
12. When the 20 minutes of gel running time is up, turn off the power supply and remove the lid of the gel tank. Carefully remove the gel (still on the gel tray) and place it on the petri dish provided. Bring your gel to the gel doc that has been assigned for your usage and the technician will photograph it for you.

13. Bring your gel and the photograph back to your cubicle and use your signal card to get assistance for an invigilator to staple it in the space provided **on the Answer Sheet**.

Q1.5 **(10 points)** Your skills in running a gel will be assessed by the quality of the gel produced.

14. Based on the gel results, answer the following questions **in the Answer Sheet**:

Q1.6 **(1 point × 5 = 5 points)** Using the DNA ladder markers (in basepairs) provided below as the reference, estimate the sizes of the fragments/bands. You may draw a line across the band of your query and the size marker to do the estimation. How many fragment(s) of DNA were generated by RE1 and RE2? And what is/are the estimated size(s)? Answer using numerals.



L1: 100 bp DNA Ladder L2: 1 kb DNA Ladder

Q1.7 **(1 point)** What is the estimated size of the test DNA sample (T)? Answer using numerals.

Q1.8 **(1 point)** Based on your results, is the test DNA sample (T) larger, smaller or the same size as the empty vector? Indicate your answer with a tick (✓) in the correct box.

Q1.9 **(1 point)** Does the test DNA sample (T) contain any insert? Indicate yes with a tick (✓) and no with a cross (✗).

Q1.10 (2 points) Uncut DNA appears to move faster than any of the samples digested with RE2. Why? Indicate your answer with a tick (✓) in the correct box.

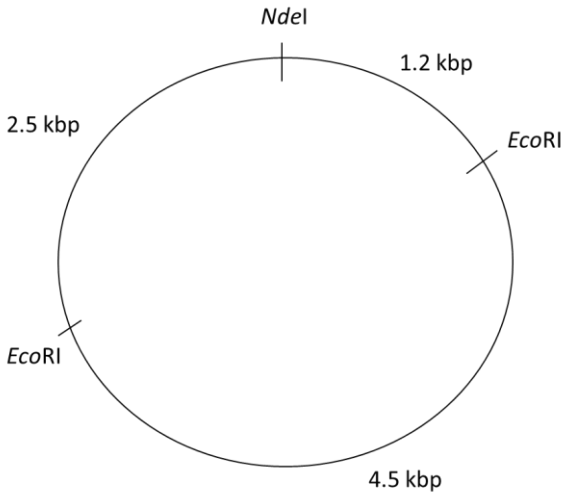
a. The smaller fragment size of uncut DNA is due to DNA degradation.

b. The uncut DNA is more compact and therefore moves faster through the gel.

c. RE2 still binds to the DNA and therefore slows down their movement through gel.

Part B. Determination of orientation by which fragment was inserted. (**20 points**)

Q1.11 **(20 points)** Construct possible restriction map(s) for the DNA "T" by indicating the relative position of RE1 and RE2 and the distance between them **in the Answer Sheet**. An example of such a map is provided below as a guide.



END OF PAPER

Country: _____	Student Code: _____

23rd INTERNATIONAL BIOLOGY OLYMPIAD

8^{th} – 15^{th} July, 2012

SINGAPORE

PRACTICAL TEST 2

MICROBIOLOGY & BIOCHEMISTRY

Total points: **100**

Duration: **90 minutes**

Dear Participants

- In this test, you have been given the following two tasks:

  Task 1: Bacteriophage: an effective agent in the killing of pathogenic bacteria. (50 points)

  Part A: Effects of Phage and antibiotics on the killing of antibiotic-resistant *E. coli* (31 points)

  Part B: Phage titre and multiplicity of infection (19 points)

  Task 2: Titration of an amino acid. (50 points)

- Use the **Answer Sheet**, which is provided separately, to answer all the questions.
- The answers written in the Question Paper will **NOT** be evaluated.
- Write your answers legibly in ink.
- Please make sure that you have received all the materials and equipment listed for each task. If any of these items are missing, please raise your hand **immediately**.
- Stop answering and put down your pen IMMEDIATELY when the bell rings.
- At the end of the test, place the Answer Sheets and Question paper in the envelope provided. Our Assistants will collect the envelope from you.

Have fun and Good Luck! ☺

Materials and equipment:

For Task I: Bacteriophage: an effective agent in the killing of bacteria

Materials and equipment	**Quantity**	**Unit**
micropipette tips 10μl	1	box
micropipette tips 200μl	1	box
micropipette tips 1000μl	1	box
micropipette 1 - 10μl	1	piece
micropipette 2 - 20μl	1	piece
micropipette 20 - 200μl	1	piece
micropipette 100 - 1000μl	1	piece
microfuge tube rack	1	piece
cuvette rack	1	piece
microfuge tubes (in a beaker)	many	tube
stock *E. coli* culture (1×10^7 cells/ml) in LB broth	1	tube
LB broth (in a 50 ml Falcon tube)	1	tube
sterile deionized water in microfuge tube	1	tube
ampicillin stock (1 mg/ml) dissolved in deionized water	1	tube
bacteriophage stock (10^8 pfu/ml) in deionized water	1	tube
cuvettes (in a beaker)	4	piece
stopwatch	1	piece
floating rack (labelled with your country code)	1	piece
water-bath 37 ºC (there is one assigned for your usage)	1	set
UV-VIS Spectrophotometer (there is one assigned for your usage)	1	set
photographs of *E. coli* plates (A to H)	1	set

For Task II: Titration of an amino acid

Materials and equipment	**Quantity**	**Unit**
25 ml burette	1	piece
25 ml pipette	1	piece
100 ml beakers	3	piece
magnetic stirring bar	1	piece
magnetic stirrer	1	set

pH meter with electrode	1	set
pipette bulb	1	piece
Kimwipe papers	1	box
retort stand with clamps	1	set
0.3024 M standardized NaOH solution	100	ml
Amino acid Z solution of unknown concentration	80	ml

Task I (50 points)

Bacteriophage: an effective agent in the killing of bacteria

Part A. Effects of Phage and antibiotics on the killing of antibiotic-resistant *E. coli* (31 points)

Introduction

A bacteriophage is a virus that infects bacterial cells. Certain bacteriophages can kill bacteria cells by lysis. The bacteriophage is now recognized as an effective agent in the killing of pathogenic bacteria. This provides a good alternative to antibiotics in our combat against disease-causing bacteria that might be resistant to traditional antibiotics. You are required to design a simple experiment, with proper controls, to examine the killing efficiency of phage of an ampicillin-resistant *E. coli*. Answer the following questions **in the Answer Sheet** and follow the instructions given below.

Q1.1 **(1 point)** To dilute the *E. coli* culture from 1×10^7 cells/ml to 2×10^5 cells/ml, what would be the dilution factor needed?

Q1.2 **(1 point)** For 1 ml of *E coli* culture at a cell density of 2×10^5 cells/ml, the final concentration of ampicillin used should be 10 μg/ml. What would be the volume of ampicillin stock (1mg/ml) used?

Q1.3 **(1 point)** For 1 ml of *E coli* culture at a cell density of 2×10^5 cells/ml, final titre of phage used should be 10^6 pfu/ml. What would be the volume of phage stock (10^8pfu/ml) used?

Q1.4 **(1 point × 15 = 15 points)** With the above calculated dilution factors, fill in the table **in the Answer Sheet** with your experimental plan. One example is already given for Tube 1 in the table provided. All units are in μl. Carry out your proposed experiment by incubating the four tubes (placed in the labelled floating rack) for 40 minutes (stop watch provided) in the 37

ºC water bath assigned to you. Hand over the floating rack to the technician at the water bath.

After incubation, transfer your samples to the cuvettes labelled 1 to 4. In order to observe the killing of bacteria cells, measure the absorbance at 595 nm wavelength. Bring your samples to the spectrophotometer that is allocated for your use and hand over your samples to the technician. You are to record your own readings as the samples are measured.

Q1.5 **(0.75 × 2 + 1.5 points × 6 = 10.5 points)** Fill in the absorbance reading at 595 nm of the different tubes of reactions in the table provided **in the Answer Sheet**. Taking 1 absorbance unit of the *E. coli* cells at 595 nm to be equivalent to 1×10^7 cells/ml, what are the cell densities of the *E. coli* in the respective reaction tubes?

Q1.6 **(0.5 points × 5 = 2.5 points)** Which of the following are correct? Indicate correct answer(s) with a tick (✓) and incorrect answer(s) with a cross (×).

a. Due to the ampicillin resistance, the bacteria cell wall prevented easy penetration of the antibiotics, but allowed the phages to enter the *E. coli* cells to cause lysis.

b. The ampicillin resistance in the *E. coli* did not prevent the ability of the phage to adsorp onto the bacteria cells.

c. The bacteriophage likely has a lytic life cycle of around 20 to 30 mins and hence lysis of the *E. coli* was observable during the short experiment.

d. The temperature of 37 ºC was not the correct temperature for ampicillin to kill the *E. coli* cells.

e. The phages competed with the *E. coli* for the nutrients in the LB broth and the bacteria cells lysed due to insufficient nutrients.

Part B. Phage titre and multiplicity of infection (19 points)

The Table below shows the legends for photographs of *E. coli* lawns that are untreated and infected with bacteriophages. The *E. coli* culture used had a starting cell density of 0.5×10^4 cells/ml. 0.5 ml of phage was used to infect the *E. coli* cells. Serial dilutions of the phage culture were made as indicated and used for infection. (The photographs labelled A to H will be provided as part of materials for the lab task).

A = 10^{-6} dilution	B = 10^{-5} dilution
C = 10^{-4} dilution	D = 10^{-3} dilution
E = 10^{-2} dilution	F = 10^{-1} dilution
G = neat phage	H = *E. coli* lawn uninfected by phage

Q1.7 **(2 points × 4 = 8 points)** Based on the number of plaques observed in the photos, calculate the number of plaques that would be observed if the original undiluted phage culture were used.

Q1.8 **(3 points)** To estimate the titre of a phage culture, serial dilutions as shown in the photos (A to H) are normally performed. Based on the number of plaques shown, indicate with a tick (✓) which is the best dilution to confirm the phage titre.

Q1.9 **(4 points × 2 = 8 points)** Using the information given and your answers above, determine:

a. the plaque forming units per milliliter (pfu/ml) of the phage culture used and

b. the multiplicity of infection(defined as the ratio of phages to *E. coli*),

at the best dilution determined in Q1.8.

Task II (50 points)

Titration of an Amino Acid

Introduction

Amino acids are organic molecules possessing both carboxyl and amino groups. Table 1 shows the 20 amino acids that cells use to build their thousands of proteins. The majority of the standard amino acids are diprotic molecules since they have two dissociable protons: one on the amino group and the other on the carboxyl group; there is no dissociable proton in the R group.

Recall: For an acid HA, the acid dissociation constant for the equilibrium of $HA \rightleftharpoons H^+ + A^-$ is K_a.

$$K_a = [H^+][A^-] / [HA]$$

More often, the strength of acids is expressed in terms of the pK_a of the acid:

$$pK_a = -\log K_a$$

In the titration of such a diprotic amino acid, the titration will thus occur in two steps as the more acidic carboxyl group (lower pK_{a1}) and the less acidic amino group (pK_{a2}) successively lose their protons.

In addition, the pH at which the net charge on the molecule is zero is called the isoelectric point (pI) of the molecule, a useful constant in characterizing and purifying molecules. Using a titration curve, the pI can be empirically determined as the inflection point between the pK_a's of the anionic and cationic forms.

The apparent pK_a values for the two dissociation steps may be extrapolated from the midpoints of each step. This can be shown by the Henderson-Hasselbalch equation:

$$pH = pK_a + \log \{ [A^-] / [HA] \}$$

The pK_{a1} (pK_a for the carboxyl acid group) is where half the acid group has been titrated. Therefore the equation becomes:

$$pH = pK_a$$

Similarly, the pK_{a2} (pK_a for the amino group) can be determined.

In this experiment, you will titrate an unknown amino acid Z and determine its pI, pK_{a1} and pK_{a2}.

Procedure

1. Fill the burette with the standardized NaOH solution. Record the exact concentration of this standardized NaOH solution **in the Answer Sheet**.
2. Pipette 25 ml of the unknown amino acid solution Z into a clean 100 ml beaker.
3. Carefully place the pH probe and a magnetic stirring bar into the amino acid solution, so that the probe is far enough into the solution, but not touching the stirring bar or beaker. Clamp and adjust the pH probe such that the stirring bar will not hit the probe while stirring. **DO NOT TOUCH THE CALIBRATION.**
4. Titration 1

   Rinse the pH probe with deionized water. Dry the probe gently with a piece of Kimwipe paper. Determine the pH of the amino acid solution Z before the addition of NaOH. Next, titrate the amino acid solution with the NaOH from the burette. Add approximately 1.00 ml of the NaOH to the amino acid at a time. Record the exact volume dispensed and the pH of the solution after every 1.00 ml interval **in the Answer Sheet**. Continue until approximately 25 ml of NaOH has been added.
5. Repeat the titration (Titration 2)

   Rinse the pH probe with deionized water. Dry the probe gently with a piece of Kimwipe paper. Refill the burette with the standardized NaOH solution and repeat steps 2 – 4.

Q2.1 **(3 points × 3 = 9 points)** Table 1 shows the chemical structures of the twenty standard amino acids. With reference to these structures, draw structures to show the complete dissociation of glycine, proline and asparagine.

Q2.2 **(3 points × 2 = 6 points)** For both titrations, record the volume of NaOH (ml) added during the titration and the observed pH value for the unknown amino acid.

Q2.3 **(5 points × 2 = 10 points)** Using your data, plot the graphs of each titration run (pH versus Vol. of NaOH (ml)) in Graphs 1 and 2 provided **in the Answer Sheet**.

Q2.4 **(2 points × 2 = 4 points)** From your titration curves, find the pI and label it on each graph.

Q2.4.1 **(2 points)** What is the mean pI?

Q2.5 **(4 points × 2 = 8 points)** Find and label the pK_{a1} and pK_{a2} on each graph.

Q2.5.1 (2 points × 2 = 4 points) What is the mean pK_{a1} and pK_{a2}?

Q2.6 **(5 points)** 0.9210 g of the unknown amino acid Z was dissolved in 80 ml of deionized water. Determine the molecular weight of the unknown amino acid Z. Note: In order to start with a fully protonated amino acid, HCl solution has been added. This is equivalent to 3.2 ml of the NaOH solution. To determine the actual number of moles of NaOH needed to reach the pI, subtract 3.2 ml from the volume of NaOH used to reach the first end point.

Q2.7 **(2 points)** Based on Table 2, identify amino acid Z.

a. glycine

b. proline

c. asparagine

d. tyrosine

e. tryptophan

Table 1. Structures of the 20 standard amino acids.

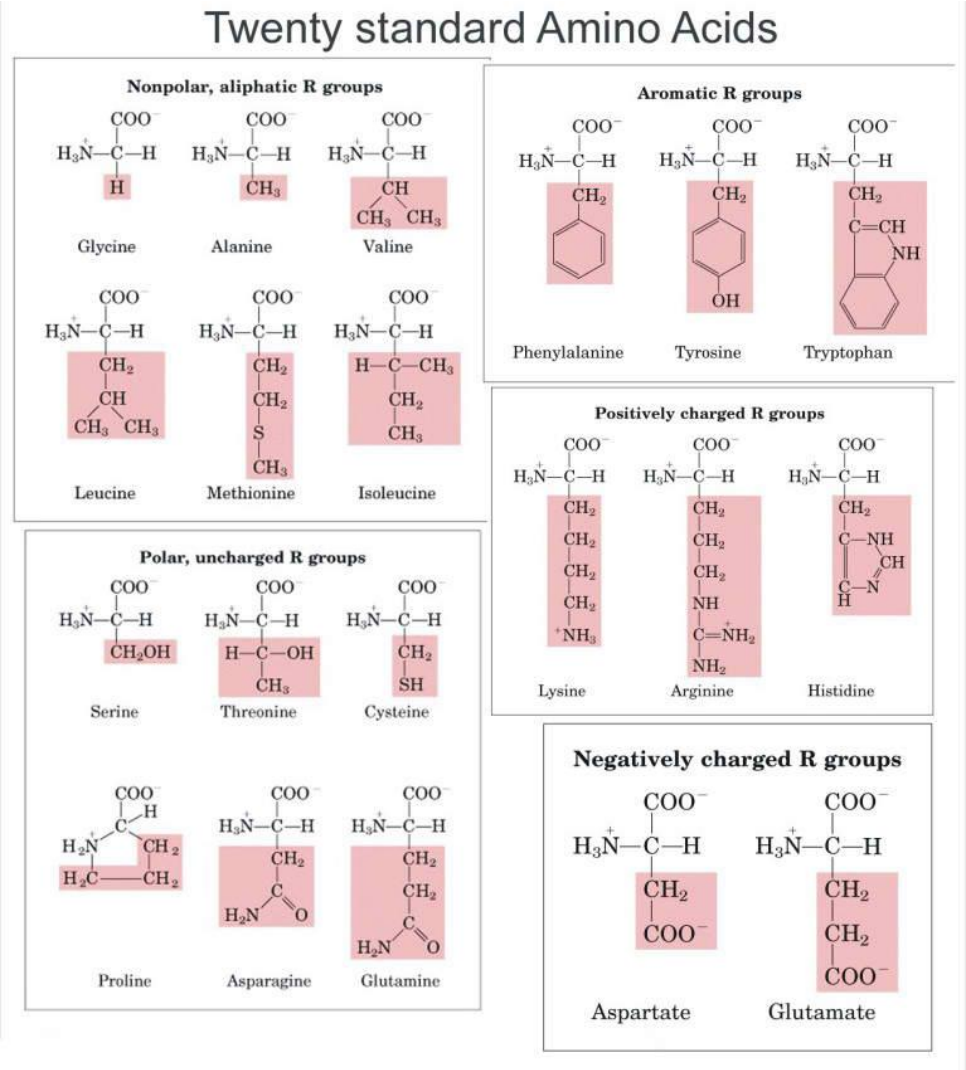


Table 2. Molecular weights of amino acids

Amino acid	MW (g/mole)
Glycine	75
Proline	115
Asparagine	132
Tyrosine	181
Tryptophan	204

END OF PAPER

Country: _____	Student Code: _____

23rd INTERNATIONAL BIOLOGY OLYMPIAD

8th – 15th July, 2012

SINGAPORE

PRACTICAL TEST 3

PLANT DIVERSITY, ANATOMY & PHYSIOLOGY

Total points: **100**

Duration: **90 minutes**

Dear Participants

- In this test, you have been given the following two tasks:

  Task I: Plant diversity and anatomy. (60 points)

  Part A: Morphology of seedlings (14.25 points)

  Part B: Seed morphology and anatomy (27.25 points)

  Part C: *Ficus* propagule dissection (5 points)

  Part D: Functional, ecological and phylogenetic aspects of seeds and seedlings (13.5 points)

  Task II: Plant anatomy and physiology. (40 points)

  Part A: Anatomy of a plant stem (13 points)

  Part B: Study of leaf epidermis and physiology (15 points)

  Part C: Interpretation of photosynthetic data (12 points)

- Use the **Answer Sheet**, which is provided separately, to answer all the questions.
- The answers written in the Question Paper will **NOT** be evaluated.
- Write your answers legibly in ink (you may use a pencil for diagrams).
- Please make sure that you have received all the materials and equipment listed for each task. If any of these items are missing, please raise your hand **immediately**.
- Stop answering and put down your pen IMMEDIATELY when the bell rings.
- At the end of the test, place the Answer Sheets and Question paper in the envelope provided. Our Assistants will collect the envelope from you.

Have fun and Good Luck! ☺

Materials and equipment:

For Task I: Plant diversity and anatomy

Materials and equipment	**Quantity**	**Unit**
Seedlings: A, B, C and D (in plastic cups)	1	specimen
Seeds/propagules: 1 to 7 (in labelled plastic bags)	1	specimen
Specimen E (in labelled plastic bag)	1	specimen
razor blade (use ONLY for Seed 3 and Seed 5)	1	piece
Scissors (use for Seed 4 and Specimen E)	1	pair
hand lens	1	piece

For Task II: Plant anatomy and physiology

Materials and equipment	**Quantity**	**Unit**
Leaves, L (in petri dish L)	2	piece
Stems, S (in petri dish S)	2	piece
concentrated HCl (in bottle labelled H, placed within a beaker)	1	bottle
Phloroglucin stain (in bottle labelled P)	1	bottle
water (in wash bottle)	1	bottle
filter paper	2	sheet
forceps	1	pair
razor blade	2	piece
plastic dropper	1	piece
petri dish (with water, labelled W)	1	piece
petri dish (labelled LL, LU and SS with the correct student code)	3	piece
beaker (small)	1	piece
glass slides	3	piece
cover slips	3	piece
compound microscope	1	set

Task I (60 points)

Plant diversity and anatomy

Part A. Morphology of seedlings (14.25 points)

Q1.1 **(0.5 points × 20 = 10 points; 2 points for quality of drawing; 2.25 points for not damaging specimens)** Make a simple schematic diagram of each seedling (A – D) in the corresponding space provided **in the Answer Sheet** and label the following (if present) with a to e and indicate if any are absent:

a. cotyledons

b. epicotyl

c. hypocotyl

d. leaves

e. seed coat

Part B. Seed morphology and anatomy (27.25 points)

Each seedling (A to D) from Part A comes from seeds 1-4 respectively.

Q1.2 **(0.25 points × 11 = 2.75 points)** Draw each whole seed (1 – 6) in the corresponding space provided **in the Answer Sheet** (Note: (1) you do not need to draw Seed 7; (2) seed coat of Seed 5 has been removed). Label the following (if present) with a and b and indicate if any part is absent:

a. seed coat

b. site of attachment of funiculus

Q1.3 **(0.5 points × 24 = 12 points; 1 point for quality of drawing)** After drawing the exterior of each seed, dissect in longitudinal section and draw the sectioned seed in the corresponding space provided **in the Answer Sheet**. (Note: Some seeds have been pre-cut for your convenience. The seed coat of Seed 5 has been removed.). Label/indicate the following items on your diagram with a to d:

a. cotyledons

b. food storage

c. hypocotyl

d. seed coat

Q1.4 **(0.5 points × 23 = 11.5 points)** Indicate the likely ploidy (1N, 2N or 3N) of each of the items labelled "a" to "d" in Q1.3 **in the Answer Sheet.**

Part C. *Ficus* propagule (dispersal unit) (5 points)

Q1.5 **(1 + 1 + 3 points)** Dissect Specimen E longitudinally and draw the longitudinal section of the propagule (dispersal unit). Draw and label an enlarged section to show in detail the features of a to c.

a. fruit

b. seed

c. stigma

Part D. Functional, ecological and phylogenetic aspects of seeds and seedlings (13.5 points)

Q1.6 **(0.5 points × 9 = 4.5 points)** Based on the observations in Parts A, B and C, and the information given in the Table provided **in the Answer Sheet**, indicate the primary function of the cotyledon with P (for photosynthesis) or S (for storage of nutrients) and the probable

germination pattern with O (orthodox: seed can undergo dormancy) or R (recalcitrant: seed does not undergo dormancy).

Q1.7 **(1 point × 5 = 5 points)** Based on the information in the Table and the diagrams that you have drawn, determine if each of the following statements is true (✓) or false (✗).

a. All tropical plants have recalcitrant seeds.

b. Gymnosperms have at most two cotyledons.

c. Cotyledon function is phylogenetically constrained within plant families.

d. Seed size varies considerably in plant families and is probably not phylogenetically constrained.

e. Large seed size may be advantageous for some tropical rainforest plants.

Q1.8 **(1 point × 4 = 4 points)** The phylogenies of Malvaceae and Moraceae, and a simplified phylogeny of seed plants are provided on the next page. Using this information, as well as the specimens that you have observed today, determine if each of the following statements is true (✓) or false (✗), or if there is insufficient evidence to conclude (–) **in the Answer Sheet.**

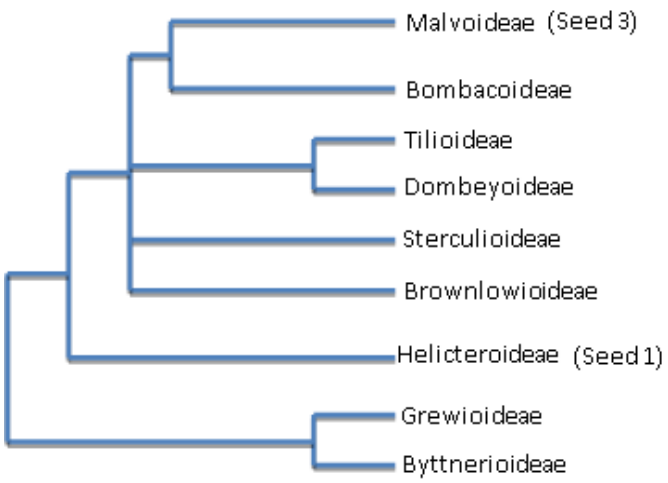
a. Large seeds have evolved independently in multiple lineages.

b. Large seeds have evolved on more than one occasion in some lineages.

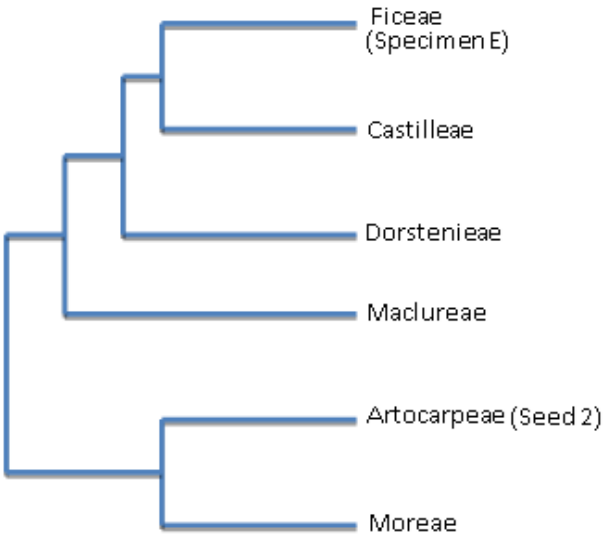
c. Recalcitrant seeds are more associated with tropical plants than with temperate plants.

d. Gymnosperms are unable to produce fleshy structures associated with animal dispersal because they lack ovaries.

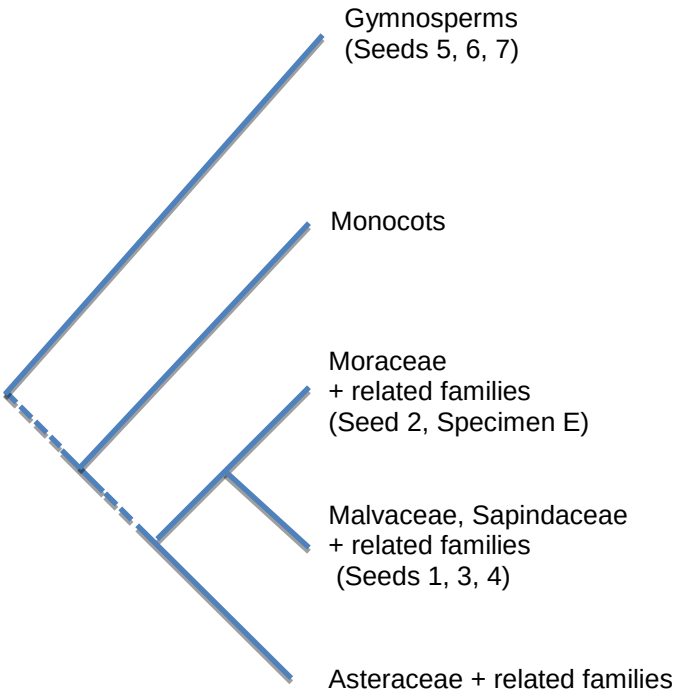
Phylogeny of Malvaceae



Phylogeny of Moraceae



Simplified phylogeny of seed plants



Task II (40 points)

Plant anatomy and physiology

Part A. Anatomy of a plant stem (13 points)

Procedure:

1. Using the razor blade, cut as thin as possible transverse sections of the stem, S.
2. Float the cross-sections in water in petri dish, W.
3. Place a drop of water onto a glass slide and transfer the best cross-section to the centre of the slide.
4. Place a small drop of phloroglucin stain (P) onto the cross-section, followed by a small drop of concentrated hydrochloric acid, HCl (H). Be very careful when handling H as it is corrosive.
5. Leave for 1 minute and then remove excess stain with the filter paper.
6. Cover the cross-section with a coverslip and examine it using the compound microscope under low magnification (4× objective lens).

Answer the following questions **in the Answer Sheet**:

Q2.1 **(1 point)** Is S a monocot stem or dicot stem? Indicate M for monocot and D for dicot.

Q2.2 **(1 point)** Is there any pith? Indicate presence of pith with a tick (✓) and absence with a cross (✗).

Q2.3 **(1 point)** Where is the vascular bundle in the stem located? Indicate centre with C and periphery with P.

Q2.4 **(0.5 points × 3 = 1.5 points)** Based on your observations, is S the stem of a shrub, tree, or herb? Indicate correct answer(s) with a tick (✓) and incorrect answer(s) with a cross (×) **in the Answer Sheet**.

Q2.5 **(0.5 points)** Identify the tissue (a – e) that is stained red. Indicate the correct answer with a tick (✓) **in the Answer Sheet**.

a. cortex

b. endodermis

c. epidermis

d. phloem

e. xylem

7. When you have completed Part A, place your slide with the stem section into the petri dish SS for grading purposes.

Q2.6 **(8 points)**

Part B. Study of leaf epidermis and physiology (15 points)

(i) Lower epidermis

Procedure:

1. Peel off the lower epidermis of the leaf, L, with a pair of forceps.
2. Place it in a drop of water on a glass slide and cover the peeled layer with a cover slip.
3. Examine it using the compound microscope under10× objective lens.

Answer the following questions **in the Answer Sheet**:

Q2.7 **(2 points)** Do you see any stomata? Indicate presence of stomata with a tick (✓) and absence with a cross (✗).

Q2.8 **(3 points)** Measure the lengths and widths of FIVE (5) epidermal cells that are representative of the majority of the cells. Smallest unit in the eyepiece micrometer as seen under 10× objective lens is 10 μm. Calculate the mean values and fill in your answer in the table provided.

4. When you have completed Part B (i), place your slide with the epidermal peel into the petri dish LL for checking purposes. The slide will also be used to check for accuracy of your measurement of epidermal cell dimensions.

(ii) Upper epidermis

Procedure:

1. Peel off the upper epidermis of the leaf, L, with a pair of forceps. You may either use the same leaf as before or a fresh leaf from petri dish L.
2. Place it in a drop of water on a glass slide and cover the peeled layer with a cover slip.
3. Examine it using the compound microscope under 10× objective lens.

Answer the following questions **in the Answer Sheet**:

Q2.9 **(2 points)** Do you see any stomata? Indicate presence of stomata with a tick (✓) and absence with a cross (✗).

Q2.10 **(3 points)** Measure the lengths and widths of FIVE (5) epidermal cells that are representative of the majority of the cells. Smallest unit in the eyepiece micrometer as seen under 10× objective lens is 10 μm. Calculate the mean values and fill in your answers in the table provided.

4. When you have completed Part B (ii), place your slide with the epidermal peel into the petri dish LU for checking purposes. The slide will also be used to check for accuracy of your measurement of epidermal cell dimensions.

Q2.11 **(0.5 point × 3 = 1.5 points)** Based on your observations in Part B (i) and (ii), indicate the correct answer(s) with a tick (✓) and incorrect answer(s) with a cross (✗).

a. There are more stomata in the lower epidermis than in the upper epidermis.
b. Epidermal cells of the upper epidermis are smaller than those of the lower epidermis.
c. Stomata are separated from each other by at least one cell.

Q2.12 **(1 point)** Based on your observations, determine what type of plant this is. Indicate the correct answer with a tick (✓) **in the Answer Sheet**.

a. hydrophyte
b. mesophyte
c. xerophyte

Q2.13 **(0.5 point × 5 = 2.5 points)** Some statements about stomatal structure, function and development are given below. Indicate true statement(s) with a tick (✓) and false statement(s) with a cross (✗).

a. Stomata consist of a pair of highly specialized guard cells that are usually surrounded by a pair of larger subsidiary cells.

b. Guard cells differ significantly from other epidermal cells in that they have chloroplasts.

c. Chloroplasts of guard cells differ from mesophyll chloroplasts in that they lack grana.

d. The number of stomata on any leaf surface is under genetic control and is not modified by any environmental factors.

e. Stomatal development involves asymmetric cell divisions.

Part C. Interpretation of photosynthetic data from plants measured at different CO_2 concentrations (12 points)

Introduction

Single leaves from plants A and B that had been grown under full sunlight in the same greenhouse were studied. The responses of their net photosynthetic CO_2 assimilation rates to varying levels of ambient CO_2 under saturating light intensity of 1,200 µmol quanta m^{-2} s^{-1} at 25 ºC and 21% O_2 measured on leaves in the laboratory are given on the next page:

Ambient CO_2 concentration (µl l^{-1})	Net photosynthetic CO_2 assimilation rate (µmol $CO_2\, m^{-2}\, s^{-1}$)	
	Plant A	Plant B
20	0.5	-4
40	11	-1
60	19	2.5
80	28	5.5
100	33	9
180	41	18
300	44	27
400	44	32
600	44	40
800	44	44
1000	44	45.5

Q2.14 (4 points). Plot a graph by using the data above for Plant A and B in Graph 1 provided **in the Answer Sheet**. Use an X-axis scale from 0 to 1000 µl l^{-1}.

Based on Graph 1, answer the following questions **in the Answer Sheet**:

Q2.15 (1 point) Indicate whether plants A and B are C_3 or C_4 plants. Indicate the correct answers with a tick (✓) **in the Answer Sheet**.

Q2.16 (2 points) What is the net photosynthetic CO_2 assimilation rate for Plant A and Plant B measured at 200 µl l^{-1} of CO_2 concentration?

Q2.17 (2 points) Plot another graph by using CO_2 assimilation rate from 20 to 100 µl l^{-1} of CO_2 concentration only (i.e., at low CO_2 concentrations) for Plant B in Graph 2 provided **in the Answer Sheet**. Use an X-axis scale from 0 to 100 µl l^{-1}.

Q2.18 (1 point) Based on Graph 2, what is the CO_2 compensation point for plant B? Write the value **in the Answer Sheet.**

Q2.19 (1 point) Compared to the data in Graph 2, would the CO_2 compensation point increase, decrease or remain unchanged if the measurements were carried out at 35 ºC and 21% O_2? Indicate the correct answer(s) with a tick (✓) **in the Answer Sheet**.

Q2.20 (1 point). Compared to the data in Graph 2, would the CO_2 compensation point increase, decrease or remain unchanged if the measurements were carried out at 25 ºC and 2% O_2? Indicate the correct answer(s) with a tick (✓) **in the Answer Sheet**.

END OF PAPER

Country: _____	Student Code: _____

23rd INTERNATIONAL BIOLOGY OLYMPIAD

8th – 15th July, 2012

SINGAPORE

PRACTICAL TEST 4

ANIMAL ANATOMY & ECOLOGY

Total points: **100**

Duration: **90 minutes**

Dear Participants

- In this test, you have been given the following two tasks:

  Task I: Anatomy of molluscs. (20 points)

  Task II: Rank-abundance plots, ABC curves and community structure. (80 points)

- Use the **Answer Sheet**, which is provided separately, to answer all the questions.
- The answers written in the Question Paper will **NOT** be evaluated.
- Write your answers legibly in ink.
- Please make sure that you have received all the materials and equipment listed for each task.

  If any of these items are missing, please raise your hand **immediately**.
- Stop answering and put down your pen IMMEDIATELY when the bell rings.
- At the end of the test, place the Answer Sheet and Question paper in the envelope provided.

  Our Assistants will collect the envelope from you.

Have fun and Good Luck! ☺

Materials and equipment:

For Task I: Anatomy of molluscs

Materials and equipment	**Quantity**	**Unit**
Mollusc 1 (in vial)	1	specimen
Mollusc 2 (in vial)	1	specimen
stereomicroscope	1	set
scissors	1	pair
forceps	3	pairs
plastic tray	2	piece
water (in beaker)	1000	mL
paper towels	4	sheet

For Task II: Rank-abundance plots, ABC curves and community structure

Materials	**Quantity**	**Unit**
Community 1	1	bag
Community 2	1	bag
Table A	1	sheet
Table B	1	sheet

Task I (20 points)

Anatomy of molluscs

Introduction

Members of the class Bivalvia are successful molluscs with a long evolutionary history. They possess hinged left and right shell valves that enclose a headless animal within.

Vials labelled 1 and 2 contain two species of marine bivalves that are common in tropical Asia but live in different habitats. The specimens were partly boiled and preserved in 70% ethanol.

Follow the instructions below to open the animals up for detailed examination under the stereomicroscope.

- Locate the anterior (if present) and posterior adductor muscles that join the left and right valves of the animal.
- Use the pair of scissors to cut the adductor muscles so that the valves can be separated to expose the internal parts of the animal.
- Observe the specimens under water in the trays provided.

Answer the following questions **in the Answer Sheet**:

Q1.1 **(3 points × 2 = 6 points)** In which habitat (a – d) would you expect to find species 1 and 2 respectively?

a. attached to rocks or other hard surfaces

b. boring into coral

c. buried in sand or mud

d. lying unattached on a sandy substratum

Q1.2 **(2 points × 2 = 4 points)** How many pairs of ctenidia (gills) are there in species 1 and 2 respectively? Answer using numerals.

Q1.3 **(2 points × 2 = 4 points)** How many pairs of labial palps are there in species 1 and 2 respectively? Answer using numerals.

Q1.4 **(2 points × 2 = 4 points)** Locate the anus near the posterior end of the animal in each species. The anus empties its contents into the path of the exhalant water flow. Starting with the anus, trace the path of the intestine forwards towards the stomach. Indicate the position of the intestine in relation to the heart (a – e) in the two species respectively.

a. intestine passes dorsally over the heart
b. intestine passes under (ventral to) the heart
c. intestine passes through the heart
d. intestine passes to the right of the heart
e. intestine passes to the left of the heart

Q1.5 **(0.4 points × 5 = 2 points)** The following is a list (a-e) of anatomical features in molluscs. Indicate with a tick (✓) if the feature may be present in bivalves and with a cross (**×**) if it is always absent.

a. crystalline style
b. eye
c. foot
d. penis
e. radula

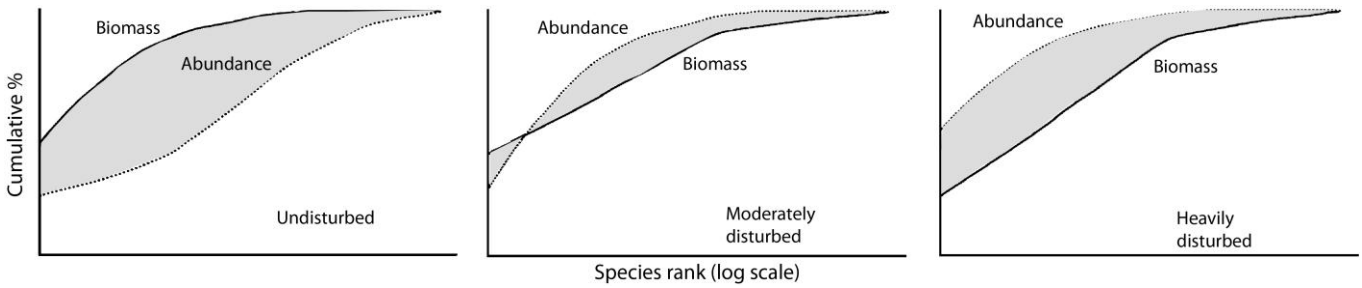
Task II (80 points)

Rank-abundance plots, ABC curves and community structure

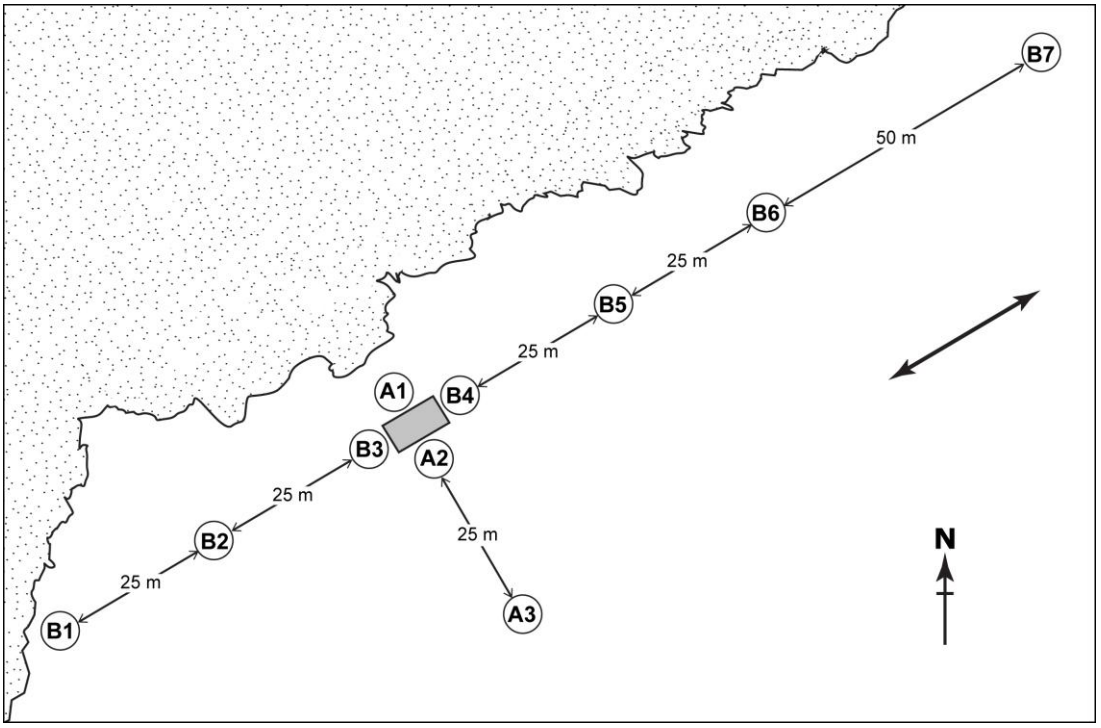
Introduction

Changes in community structure may be visualized using a variety of graphs. A rank-abundance plot or "Whittaker plot" is used by ecologists to display relative species abundance, a component of biodiversity. In this type of graph, the rank of each species is plotted along the X axis. The most abundant species is ranked 1, the second most abundant species is ranked 2, and so forth. The abundance of each species is plotted on the log scale of the Y axis. The shape of the curve can provide an indication of dominance or evenness.

The Abundance-Biomass Comparison (ABC) method was proposed by Warwick (1986) as a technique for monitoring disturbance on benthic invertebrate communities. ABC curves have a theoretical background in classical theory of *r*- and *K*-selection. The relative positions of the abundance curve and biomass curve serves to indicate the level of disturbance in the community (see graph below).



Q2.1 **(16 points × 2 = 32 points)** In an environmental impact assessment (EIA) study on the impact of salmon cage farming on benthic communities, samples were collected from the various stations along two transects, A1 to A3, and B1 to B7 (see figure below).



, location of floating salmon cages; ⟷ , indicates direction of current flow along the coast.

You are a summer intern at the marine laboratory in which this EIA study is conducted. Your responsibilities include the processing of benthic samples. You are given two bags containing Community 1 and 2 and your job is to process the samples and collate information similar to that carried out by a senior research assistant for Community 3 and 4 (see Table A, page 11 for Summary of the information). Each community may contain any of the 17 species (A – Q) listed in Table B (page 12); the respective mean fresh biomass per individual of each species is also provided in Table B.

The abundance of each species in Community 1 and 2 is indicated with different-sized chips (see photograph below); e.g., there are 61 individuals of species A shown here:

1 individual 10 individuals 50 individuals

- Determine the abundance of each species in Community 1 and record your data in Table 1 **in the Answer Sheet**.
- Fill in the rest of the required information (to 2 decimal places) in the table. Please note that lg in the table and figure represents $\log_{10}$ and in the calculator, this is represented by the log button.
- Repeat the entire procedure for Community 2.

Q2.2 **(3 points × 4 = 12 points)** Using your data in Tables 1 and 2, as well as the data provided in Tables 3 and 4, plot the rank-abundance curves for Community 1 to 4 on Graphs 1 to 4 provided **in the Answer Sheet**.

Answer the following questions **in the Answer Sheet**. Indicate correct answer(s) with a tick (✓) and incorrect answer(s) with a cross (✗).

Q2.2.1 **(1 point × 5 = 5 points)** Low evenness is:

a. indicated by a steep slope in the rank-abundance curve.

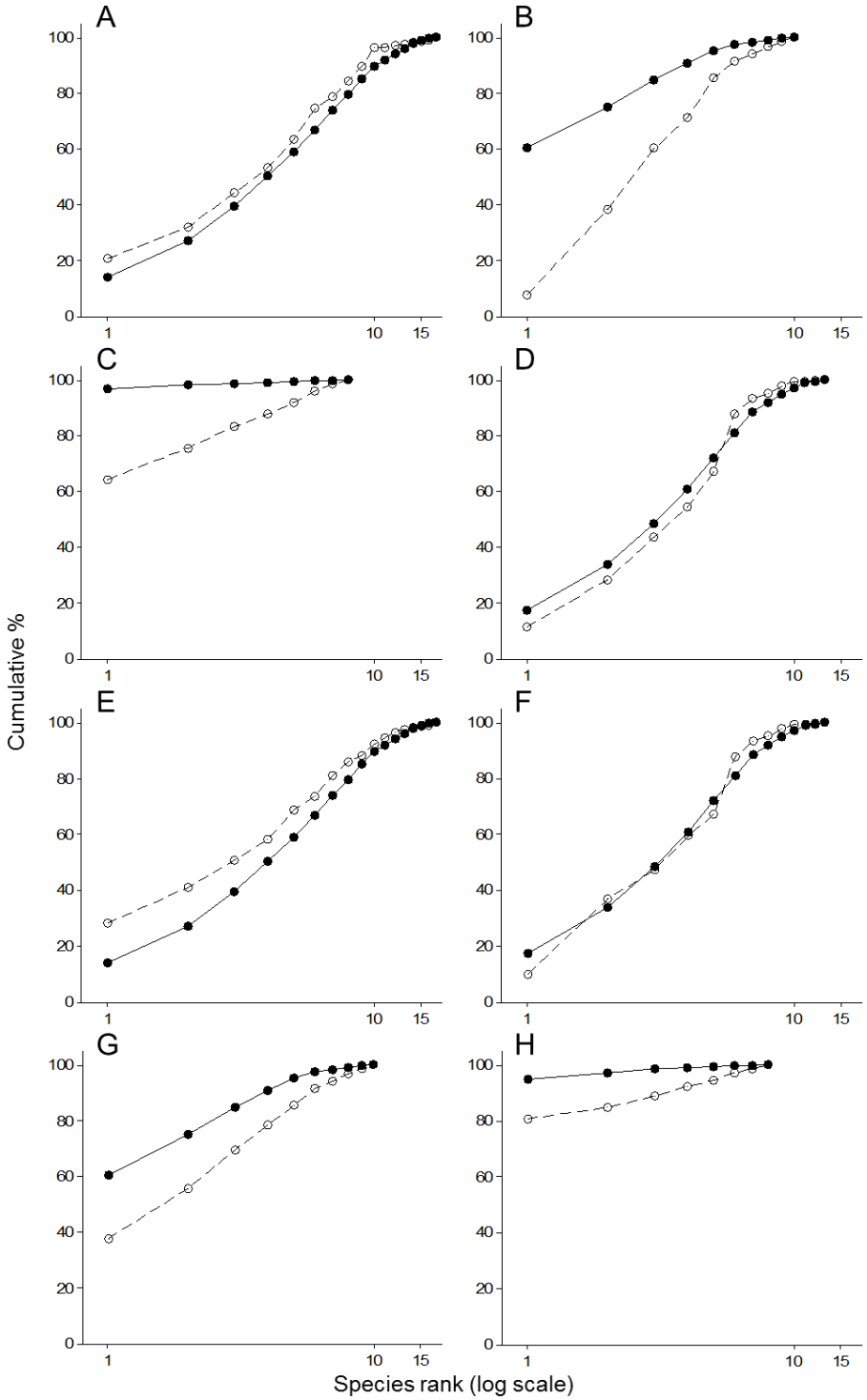
b. shown in Community 1.

c. shown in Community 2.

d. shown in Community 3.

e. shown in Community 4.

Q2.2.2 **(2.5 points × 4 = 10 points)** Indicate the correct ABC curves (A – H) that correspond to Community 1 to 4.



Q2.2.3 **(1.5 points × 4 = 6 points)** Rank Community 1 to 4 in decreasing levels of disturbance.

Q2.2.4 **(1 point × 10 = 10 points)** A careless summer intern, mixed up the labels for the sampling stations (A1 – A3; B1 – B7) when the samples were transferred from leaking containers to new bottles. From which stations could the benthic samples containing Community 1 to 4 likely to be collected?

Q2.2.5 **(2.5 points)** Which of the 17 species (A – Q) is likely to be a bivalve?

Q2.2.6 **(2.5 points)** Which of the17 species (A – Q) has the potential to be a bioindicator of organic enrichment?

Table A

Community 3

Species	Abundance	Rank	Log_{10} (lg) Abundance	Cumulative % Abundance	Cumulative % Biomass
D	200	1	2.30	14.31	28.30
C	180	2	2.26	27.18	41.03
L	175	3	2.24	39.70	50.76
N	150	4	2.18	50.43	58.34
P	120	5	2.08	59.01	68.65
B	112	6	2.05	67.02	73.74
M	98	7	1.99	74.03	81.17
Q	80	8	1.90	79.76	86.02
O	75	9	1.88	85.12	88.29
E	62	10	1.79	89.56	92.36
F	35	11	1.54	92.06	94.40
H	30	12	1.48	94.21	96.45
A	28	13	1.45	96.21	97.44
G	25	14	1.40	98.00	98.45
J	15	15	1.18	99.07	98.48
I	8	16	0.90	99.64	98.99
K	5	17	0.70	100.00	100.00
Total	**1398**				

Community 4

Species	Abundance	Rank	Log_{10} (lg) Abundance	Cumulative % Abundance	Cumulative % Biomass
J	320	1	2.51	60.49	7.83
G	78	2	1.89	75.24	38.37
B	50	3	1.70	84.69	60.39
A	32	4	1.51	90.74	71.35
F	25	5	1.40	95.46	85.42
I	10	6	1.00	97.35	91.53
N	5	7	0.70	98.30	93.98
H	4	8	0.60	99.05	96.62
E	3	9	0.48	99.62	98.53
M	2	10	0.30	100.00	100.00
Total	**529**				

Table B. Fresh biomass per individual for species A to Q.

Species	Mean fresh biomass (g)
A	0.70
B	0.90
C	1.40
D	2.80
E	1.30
F	1.15
G	0.80
H	1.35
I	1.25
J	0.05
K	4.00
L	1.10
M	1.50
N	1.00
O	0.60
P	1.70
Q	1.20

END OF PAPER