Shared instruments:

Camera, spectrophotometer, printer

Equipments and Materials:

	Equipments:	**Quantity**
1	Power supply	1
2	Electrophoresis tank (with gel and buffer)	1
3	Micropipettes P20 and P200	1 each
4	80-well microcentrifuge tube rack	1
5	Wire test tube rack with 15-mL centrifuge tubes (×6) (yellow cap)	1
6	4-way test tube rack	1
7	Plastic droppers in 15-mL centrifuge tubes	2
8	Micropipette tips (for P20 and P200)	1 each
9	Timer	1
10	96-well microplate	1
11	Marker pen & paper label	1 each
12	600-mL beaker for waste disposal	1
13	Scissors and ruler	1 each
14	Double-sticker to attach the results	1
15	Student Code sticker	1
16	Tissue paper	1
17	Mini centrifuge (if you need to spin down the samples in the microcentrifuge tubes)	1

	Materials:	**Quantity**
1	Loading dye (microcentrifuge tube-L) (pink tube with orange label)	1
2	Pre-stained protein molecular weight marker (microcentrifuge tube-M) (pink tube with orange label)	1
3	Unknown pre-stained protein samples (microcentrifuge tubes-U1 and U2) (pink tube with orange label)	1
4	CBG reagent in 50-mL centrifuge tube	1

5	Bovine serum albumin (BSA) concentration standard (0.5 mg/mL) in microcentrifuge tube (green tube with yellow label)	1
6	Enzyme E in two microcentrifuge tubes: concentrations X and Y (green tube with yellow label)	1
7	Distilled water (microcentrifuge tube-ddH_2O) (green tube with yellow label)	1
8	Protein sample (microcentrifuge tube-C) (blue tube with blue label)	1
9	Anion exchange chromatography column on 15-mL centrifuge tube	1
10	Anionic buffers A and B (5 mL each in two separated 15-mL centrifuge tubes) (green cap)	1
11	Coomassie brilliant blue G-250 (CBG) reagent 1 mL in each of six 15-mL centrifuge tubes (A1 to A3 & B1 to B3, red cap)	1

Task I (35 points)

Protein electrophoresis

Introduction:

Polyacrylamide gel electrophoresis (PAGE) is a common technique for protein study. It can be used to separate different proteins based on their charges or sizes. A type of PAGE is termed SDS-PAGE, in which the negatively charged chemical, SDS, is added before protein electrophoresis. The amount of SDS that binds to proteins is proportional to the size of the protein which confers each protein a similar charge-to-mass ratio and renders the intrinsic charge of the protein insignificant, at least for this experiment. Thus, the major factor that affects the migration of protein is the molecular weight (MW) of the protein during SDS-PAGE. The relative mobility (R_f) of the protein can be calculated as the ratio of the distance migrated by the protein to that migrated by the dye-front. The value of R_f is inversely proportional to the log of its molecular weight.

In the problem set, you will perform the following experiment:

1. An electrophoresis tank has been set up for SDS-PAGE, in which a polyacrylamide gel has been secured on electrode assembly and electrophoresis buffer has been filled. There are 10 wells for sample loading on the top of the gel. To load the sample, use the P20 micropipette with tip to

withdraw protein sample, and carefully place the tip on the top of the well. By injecting slowly the sample will sink to the bottom of the well by gravity (**Figure 1**).

2. If you need to practice, use the P20 micropipette with tip to withdraw 10 μL of loading dye from microcentrifuge tube L (pink tube with orange label) on rack. Load the dye into wells 1 to 3 or 7 to 10.
3. Each of the microcentrifuge tubes M, U1 and U2 (pink tube with orange label) contains 15 μL of protein molecular weight marker, unknown protein U1 and unknown protein U2, respectively. Use micropipette P20 to withdraw 10 μL solution from each tube and load the samples into wells 4 to 6 as shown in **Figure 1**.
4. As soon as you finish sample loading, **Lift the sign**, lab assistants will connect the power cord to power supply and set the voltage to 200 V for you. The gel will run for 25 minutes. The timer will be set up by an assistant to countdown.
5. After finishing electrophoresis, **Lift the sign**, lab assistants will disassemble the electrophoresis set-up and give back your gel. Wipe clean the surface of gel with tissue papers and **label the gel with your Student Code** sticker. Lab assistants will take the photo of your gel. Put the photo on the answer sheet using double-sticker (5 points).

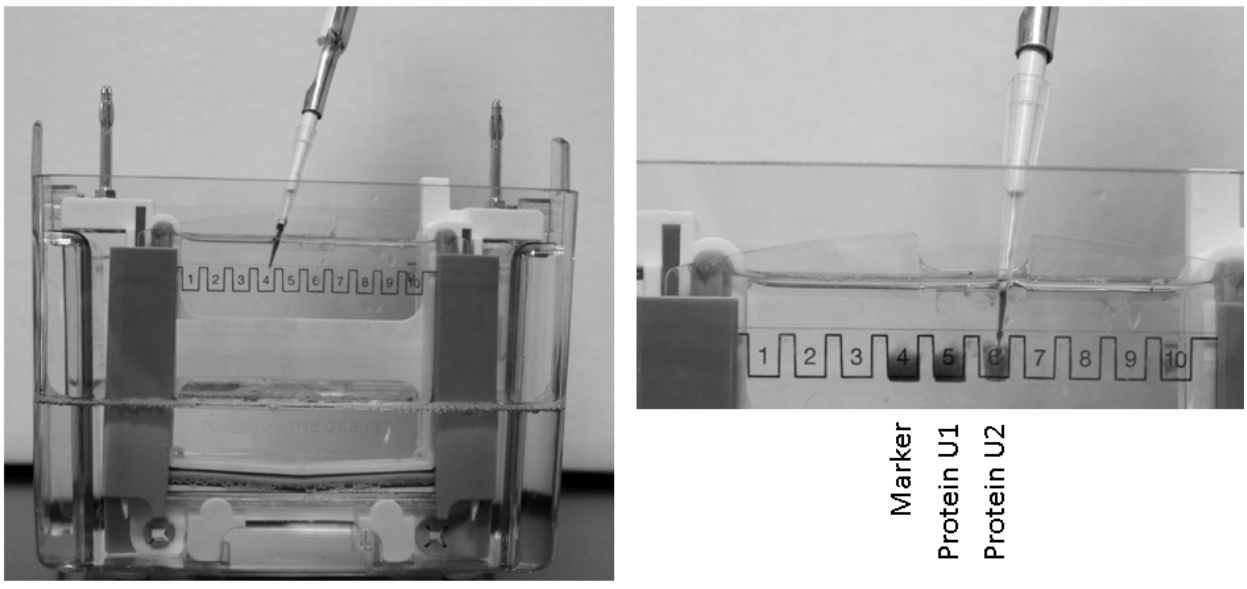


Figure 1

Answer the following questions:

Q.1.1. (2 points) **Figure 2** shows a photograph of a SDS-PAGE gel. The electrophoresis start point and dye-front are indicated. Which side of the gel should be connected to the anode (+ charge) of the power supply? Mark your answer (X) on the answer sheet.

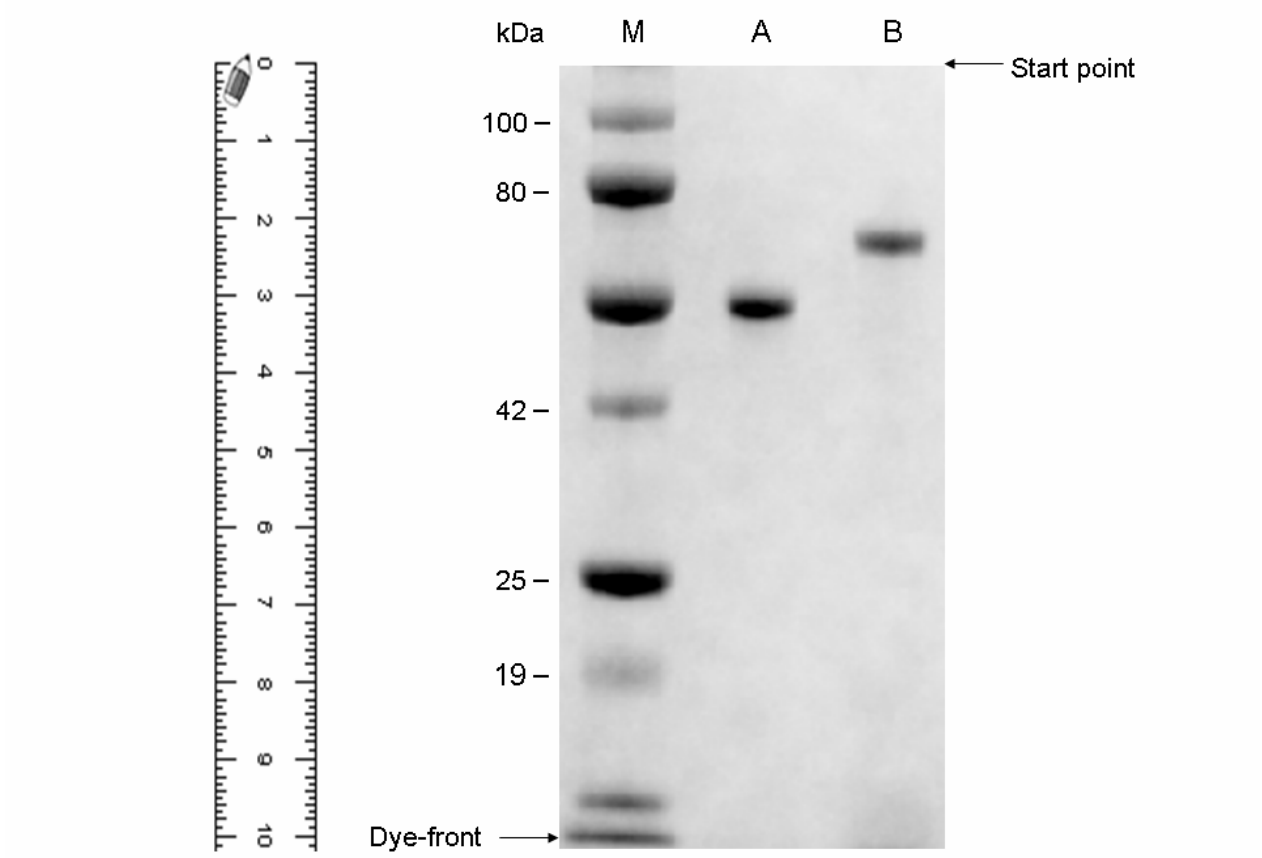


Figure 2

Q.1.2. (8 points) Based on the information provided in **Figure 2**, plot a graph of molecular weight values on graph paper provided of the five marker proteins versus their relative migration-R_f values on the answer sheet (4 points). Use the graph to estimate the molecular weights of unknown proteins on lanes A and B (4 points). Write down your answers on the answer sheet.

Q.1.3. (5 points) A protein complex of molecular weight 246 kDa is composed of multiple subunits bound by non-covalent interaction. Two protein bands of 57 and 33 kDa were identified after SDS-PAGE. How many 57-kDa and 33-kDa subunits, respectively, are included in the protein complex? Write down your answers on the answer sheet.

Q.1.4. (5 points) The average molecular weight of amino acid residues is about 110 daltons. How many amino acids are there in the 33-kDa protein subunit? How many nucleotides of RNA are translated into the protein? Write down your answers on the answer sheet.

Q.1.5. (5 points) Suppose the average molecular weight of nucleotides is 330 daltons. Excluding intron and stop codon, what is the mass ratio of dsDNA encoding the 33-kDa protein to the 33-kDa protein? Write down your answer on the answer sheet.

Q.1.6. (5 points) Suppose a protein P can bind to a protein Q (MW = 1000 daltons). The binding can be revealed by gel-mobility shift assay. Now 200 pmol of protein P were mixed with various amounts (0 to 500 ng) of protein Q. These mixtures were resolved by 10% (w/v) polyacrylamide gel. Gel was stained by Coomassie blue and is shown in **Figure 3**. Calculate the binding molar ratio of proteins P and Q? Write down your answer on the answer sheet.

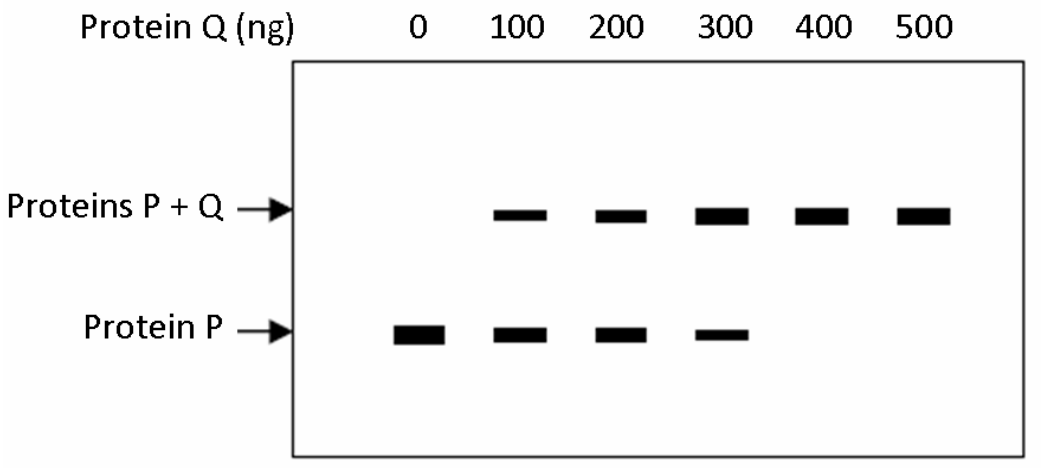


Figure 3

Task II (30 Points)

Protein quantification

Introduction:

Coomassie Brilliant Blue G-250 (CBG) is a protein staining reagent. It appears in a different color under different pH conditions. It looks reddish brown in acidic solution, whereas it turns blue under neutral or alkaline condition. Since proteins can provide a relative neutral environment, CBG will turn blue with the maximum absorbance at a wavelength of 595 nm when binding to protein. The more protein there is in a sample, the more CBG will bind to it, and thus, the higher intensity of blue color will be. In other words, the absorbance at 595 nm is proportional to the amount of protein in a sample. Based on this, one can determine the concentration of protein by measuring the blue intensity of a sample.

In the problem set, you will perform the following experiment:

1. To make BSA concentration standards (**Table 1**), add 0, 2, 4, 6, 8 and 10 μL of 0.5 mg/mL BSA (green color) in A1 to A6 wells of a microplate (**Figure 4**). Make duplicated BSA concentration standards in B1 to B6 wells. If this step is incorrect, you can repeat the procedure in wells A7 to A12 and/or B7 to B12. Adjust the total volume of each BSA solution to 10 μL by adding an appropriate volume of H_2O (**Table 1**).

2. Add 200 μL of CBG reagent per well in A1 to A6 & B1 to B6. Mix and observe the color change.

3. To determine the two concentrations X and Y of enzyme E, add various amounts (2, 4, 6, 8 and 10 μL) of enzyme E (green color) in duplicate to empty wells and bring up the volume to 10 μL with H_2O.

4. Add 200 μL of CBG reagent per well to the diluted enzyme E. Mix and observe the color change.

5. **Lift the sign**, lab assistants will accompany you to measure the absorbance values of your samples at 595 nm using spectrophotometer. Put your Student Code on the print-out data with

marker pen.

6. Return to your work bench, and put the result on the answer sheet using double-sticker.

Table 1

Materials	Well of a microplate					
	A1 & B1	**A2 & B2**	**A3 & B3**	**A4 & B4**	**A5 & B5**	**A6 & B6**
0.5 mg/mL BSA (μL)	0	2	4	6	8	10
H_2O (μL)	10	8	6	4	2	0
Diluted BSA concentration (mg/mL)	0					

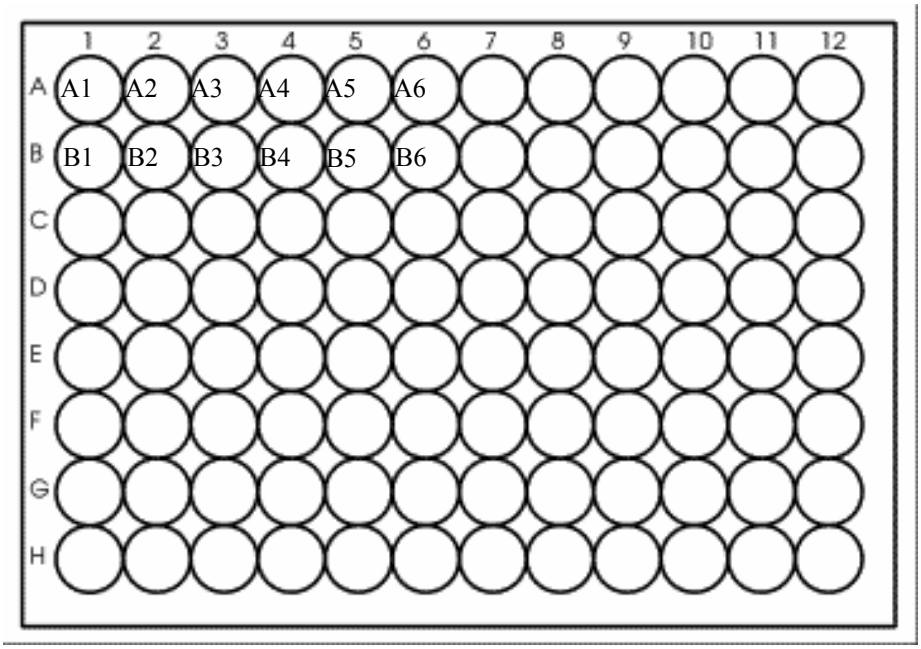


Figure 4

Answer the following questions:

Q.2.1. (10 points) Calculate the concentrations of BSA in each sample (10 μL) and fill in the blanks in the table on the answer sheet (Q2.1.1. 5points). Use these values to plot a standard curve of BSA concentrations (X-axis) versus mean absorbance values of duplicated standards (Y-axis) on the answer sheet (Q.2.1.2. 5points).

Q.2.2. (12 points) Choose the best sample solution of diluted solutions X and Y within the range of BSA standard curve and fill in the table on the answer sheet.

Q.2.3. (8 points) Based on the best sample solution you chose, calculate the original concentrations (X and Y) of enzyme E from the standard curve of BSA concentration. The concentrations should be expressed in units of mg/mL. Write down your answers on the answer sheet.

Task III (35 points)

Protein purification

Introduction:

Column chromatography is commonly used for purification of proteins. The column is made by packing solid porous material (stationary phase) in a column filled with buffer solution (mobile phase). The protein solution to be separated is loaded on top of the column and allowed to percolate into the solid matrix (stationary phase). A reservoir at the top supplies elution buffer constantly which flows through the matrix and passes out of the column at the bottom (the eluent). Since proteins interact with solid matrix in different degree, individual proteins migrate faster or more slowly through the column depending on their properties. Therefore, one can obtain purified proteins by collecting eluent at different times (**Figure 5**).

Ion-exchange chromatography can be used to separate proteins with different electric charge at a given pH. In anion exchange chromatography, negatively charged proteins bind to positively charged stationary phase. Using solution containing anions to compete with proteins for the adsorption of solid matrix, the bound proteins will be eluted. In practical, proteins are eluted first with buffer containing lower concentration of anion, then with buffer containing higher concentration of anion. Since different charged proteins interact with the stationary phase in different strength, they can be separately eluted by different concentrations of anionic buffers.

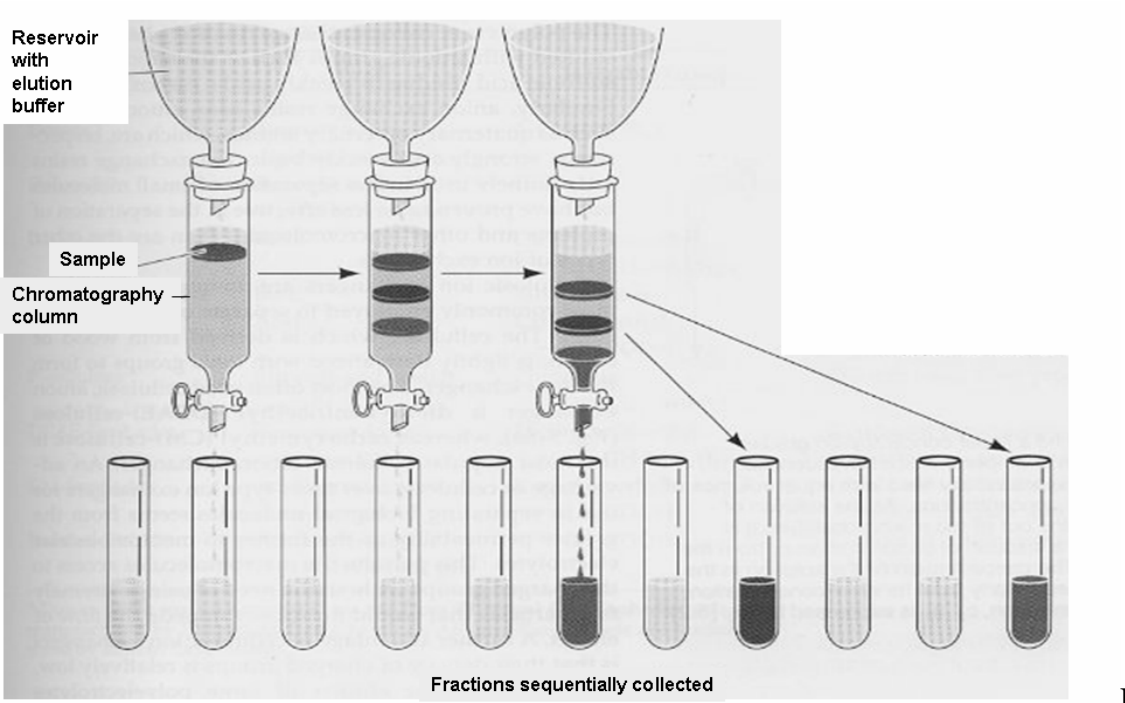


Figure 5

<u>In the problem set, you will perform the following experiment (5 points):</u>

1. Label six 15-mL centrifuge tubes (yellow cap) a1 to a3 and b1 to b3 accordingly, with a marker pen.
2. Take the anion chromatography column (**Figure 6A**), un-plug the tube and allow the solution to be drained by gravity in the same centrifuge tube. Plug the tube intermediately when the liquid surface reaches the top of the disc (**Figure 6A**, white arrow). Do not over-dry the gel as it may affect protein purification.
3. Withdraw 200 μL of protein solution from microcentrifuge tube C (blue tube with blue label) using a P200 micropipette, apply the sample to the chromatography column slowly by touching the filled pipette tip lightly against the inside wall of the tube (**Figure 6B**).
4. Un-plug the column and allow the protein sample to drain out, then transfer the column to centrifuge tube a1 (yellow cap). Withdraw 3 mL of anion buffer A (green cap) with a plastic dropper and apply the solution to gel by touching pipette tip against the wall of the tube (**Figure 6C**).
5. Collect ~1 mL eluent in centrifuge tubes a1 to a3 (yellow cap) sequentially. It takes about 2 to 3

minutes for each tube.

6. Allow the contents of the column to **drain entirely out** then transfer the column to centrifuge tube b1 (yellow cap). Withdraw 3 mL of anion buffer B (green cap) with a plastic dropper and apply the solution to gel by touching pipette tip against the wall of the tube (**Figure 6C**).
7. Collect ~1 mL eluent in centrifuge tubes b1 to b3 (yellow cap) sequentially. It takes about 2 to 3 minutes for each tube.
8. Withdraw 50 μL of eluent from tubes a1 to a3 & b1 to b3 (yellow cap) and transfer to centrifuge tubes A1 to A3 & B1 to B3 (red cap), respectively. Mix and observe color change. CBG (see introduction in Task II) reagent in tubes A1 to A3 & B1 to B3 will turn blue when it reacts with the eluted protein.
9. After finishing all the experiments, **Lift the sign**, lab assistants will take photo of your experiment results and put a stamp mark on your answer sheet. Without the stamp mark, you will not be evaluated for Q.3.1.1. and Q.3.1.2.

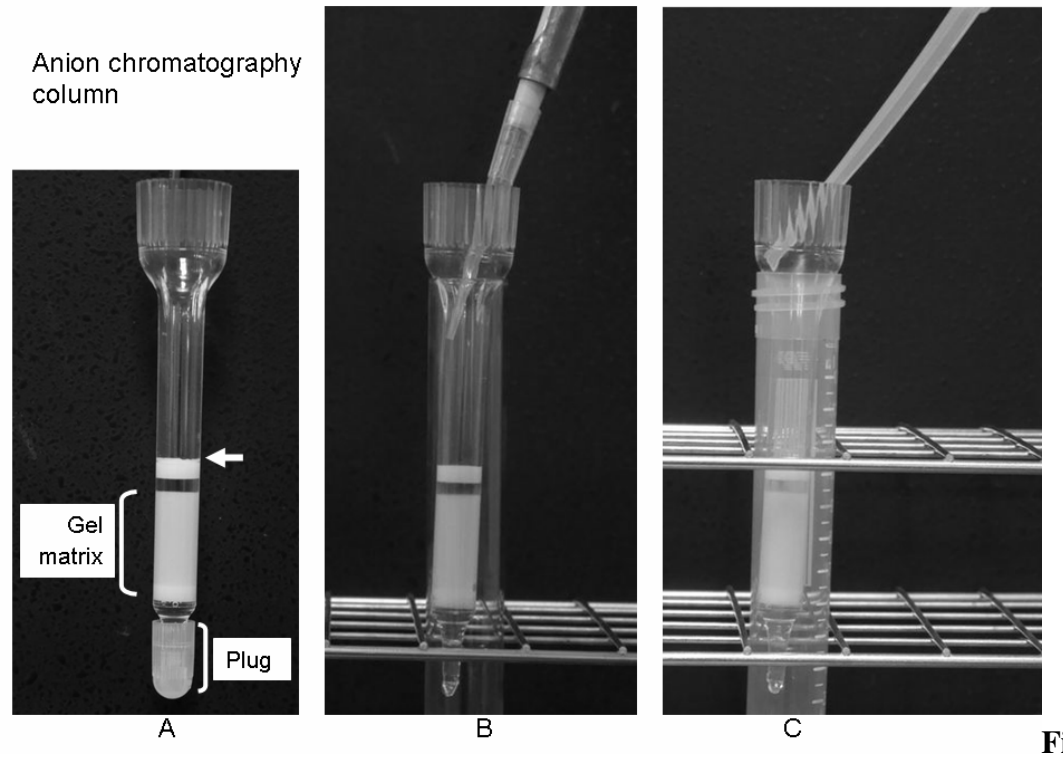


Figure 6

Q.3.1. (7 points) Mark the deepest color change (X) on the answer sheet (Q.3.1.1. 5 points). Which of the following buffers (buffer A or buffer B) can be used to elute the protein? Mark your answer (X) on the answer sheet (Q.3.1.2. 2 points).

Q.3.2. (5 points) Enzyme A is a protein whose surface is evenly distributed with electric charges. If enzyme A can be eluted from anionic exchange chromatography by high concentration of anionic buffer, what is the property of enzyme A with respect to electric charge? Mark (X) the answer on the answer sheet.

(A) High negative net charges

(B) Low negative net charges

(C) Zero net charge

(D) Low positive net charges

(E) High positive net charges

Q.3.3. (4 points) Different amino acids differ in the chemical nature of the R group (side chain). **Figure 7** shows four amino acids A, B, C, and D in their prevailing ionic forms at pH 7.2, with the side chain marked in white box. Which of the following amino acids in **Figure 7** would be present more frequently on enzyme A? Write down your answer (X) on the answer sheet.

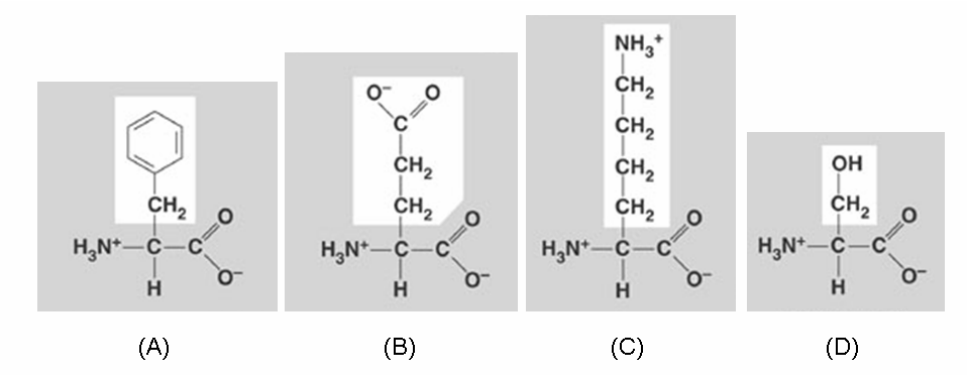


Figure 7

Q.3.4. (5 points) Hydrophobic interaction chromatography can be used to separate proteins based on their hydrophobicity. To perform the chromatography, protein samples were first treated with buffer containing high concentration of salts such as ammonium sulfate $(NH_4)_2SO_4$, which will remove water molecules from the protein surface. This causes the hydrophobic area on the surface

of the protein to be exposed. When the salt-treated proteins are subjected for chromatography, they will be absorbed on the stationary phase through hydrophobic interactions. The higher the hydrophobicity of the protein, the stronger the absorption. As salt concentration can affect the hydrophobic interaction between the protein and the stationary phase, different proteins can be separately eluted by using different concentrations of salt-containing buffers. If enzyme A is highly hydrophobic, which of the following buffers should be used to separate enzyme A from other proteins by chromatography? Mark (X) the answer on the answer sheet.

(A) Low-salt buffer

(B) High-salt buffer

(C) Buffer without salt

(D) Low-salt buffer first then high-salt buffer

(E) High-salt buffer first then low-salt buffer

Q.3.5. (4 points) If enzyme A is highly hydrophobic, which of the amino acids in **Figure 7** would be present more frequently on enzyme A? Mark (X) the answer on the answer sheet.

Q.3.6. (5 points) Gel filtration chromatography separates proteins based on their sizes. The gel, or stationary phase, consists of cross-linked polymer beads with engineered pores of a particular size. Small proteins enter the pores and are retarded by their more labyrinthine path. Large proteins cannot enter the pores and so take a short path through the column, around the beads. **Table 2** is a list of gels and their fractionation ranges. Suppose both enzyme A (22 kDa) and protein B (44 kDa) are single-subunit proteins. One would like to purify enzyme A from a mixture containing enzyme A and protein B using gel filtration chromatography. Which gel is best suited for the job? Mark your answer (X) on the answer sheet.

Table 2

Types of stationary phase	Fractionation range (MW, Da)
G-10	<700
G-15	<1500
G-25	1,000-6,000
G-50	1,500-30,000
G-75	3,000-70,000
G-100	4,000-150,000
G-150	5,000-400,000
G-200	5,000-800,000

Q.3.7. (5 points) Assume that the concentration of total proteins in the original solution is 1 mg/mL and the activity of enzyme A is 0.5 units in 1-mL protein sample. The concentration of total proteins after purification is 0.1 mg/mL and the activity of enzyme A is 1 unit in 1-mL protein sample. Calculate the purification factor (times of purity improvement) of enzyme A. Write down your answer on the answer sheet.

PRACTICAL TEST 2 : ANIMAL PHYSIOLOGY AND ANATOMY

Total Points: 100 Duration: 90 minutes

Dear Participants,

- In this test, you have been given the following 2 tasks:

  Task I: The observation of the sciatic nerve of American bullfrog (58 points)

  Task II: The observation of tissue morphology and the match of their functionality (42 points)

- Check your **Student Code** on the **Answer Sheet** before starting the test.
- Write down your results and answers in the **Answer Sheet. Answers written in the Question Paper will not be evaluated.**
- Make sure that you have received all the materials listed for each task. If any of the listed items is missing, **raise your sign.**
- **Use pen only.**
- **You must complete task I first.**
- Stop answering and put down your pen **immediately** after the end bell rings.
- After test, enclose both the **Answer sheets and Question paper** test sheets in the provided envelope. Our Lab assistants will collect it promptly.
- No paper or materials should be taken out from the laboratory.

Good Luck!!

Equipment and Materials:

For task I: The observation of the sciatic nerve of American bullfrog.

Instruments/materials	Quantity	unit
Bullfrog specimen	1	piece
Dissecting tray (containing a cooler)	1	piece
Round plastic petri dish	1	piece
Ringer's solution (in wash bottle)	500	mL
Pin (in a glass bottle)	10	pieces
Cotton line (in a glass bottle)	2	pieces
Electric stimulating device	1	set
Wet paper	1	set
Plastic glove	1	pair
Dissecting equipment : scissors (large)	1	pair
scissors (small)	1	pair
fine forceps	2	pairs

For task II: The observation of tissue morphology and the match of their functionality

Instruments/materials	Quantity	unit
Microscope	1	set
Tissue section (marked A to J)	10	slides
Color pictures (numbered 1 to 9) on 3 sheets of A4 paper	1	set

Task I (57 points)

The observation of the sciatic nerve of American bullfrog.

Introduction:

The sciatic nerve is a branch of the sacral plexus. It is the thickest and longest nerve tract in the body, extending from the vertebral column to the foot. The sciatic nerve includes the distributed sensory and motor nerves that control most sensory and motor activities of the lower extremities. Mediated by the sciatic nerve, sensory signals from the lower limbs are transmitted to the brain. Similarly, muscle contraction of the lower extremities can be stimulated by nerve impulses from the brain. The aim of this experiment is to observe and isolate the sciatic nerve from the bullfrog.

Experiment Procedure:

Step 1 to 5: (To keep the tissues wet, a small amount of Ringer's solution may be added onto the tissue anytime. Do not allow the tissues to dry out.)

1. Carefully check if all the experiment instruments/materials are fully provided. Raise your sign if you have any problem.
2. Put the bullfrog specimen on the provided dissecting tray.
3. First, carefully observe the 10 pairs of spinal nerves extending from the vertebra of the bullfrog. Next, locate the sciatic nerve that is assembled by pairs of spinal nerves VII, VIII and IX (as shown in Fig. 1).
4. Turn on the switch (on/off) on the electric stimulating device. The red light will be lighted up immediately, indicating that the device is functioning.
5. Simultaneously stimulate the sciatic nerve with the two electric wires that are separately connected to the (+) and (-) electrodes of the electric stimulating device. Fig. 3 indicates the position of sciatic nerve emerging form the spinal cord. Observe the contracting response of the

hind limb.

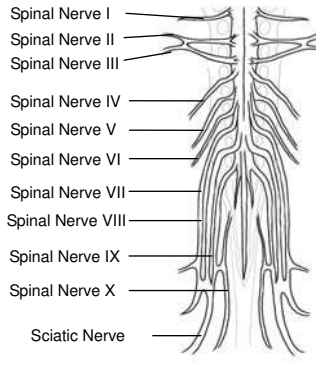


Fig. 1. Spinal nerve

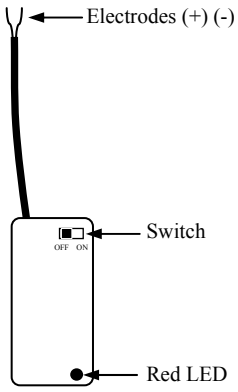


Fig. 2. Electric stimulating device

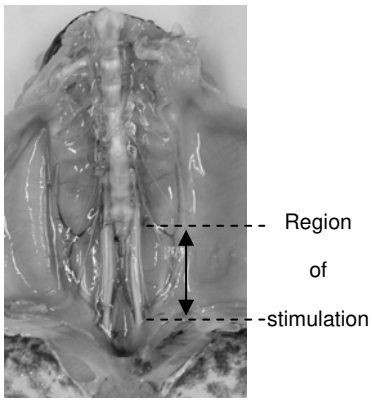


Fig. 3. Sciatic nerve

Q.1.1. (9 points) When you have finished the above five steps, **lift the sign** to notify the Lab assistant to videotape the contraction.

Step 6 to 10: (To keep the tissues wet, a small amount of Ringer's solution may be added onto the tissues any time)

6. Use a pair of scissors to circularly cut open the skin at the upper part of **one** thigh of the bullfrog. Starting from the cutting point, completely peel off the skin by hand to remove it from the hind limb (Fig. 4). It may be necessary to cut some connections between the skin and underlying tissues.

7. Lay the bullfrog on the dissecting tray with its back facing up.

8. Push two pins separately into both ends of gastrocnemius and separate it from tibiofibula (shinbone) (Fig. 5).

9. The sciatic nerve is located in the trough surrounded by thigh muscles. Carefully separate the muscles on both sides of the trough and let the light yellow colored sciatic nerve be exposed.

Pass through a cotton line underneath the sciatic nerve to label it.

10. Stimulate the cotton line-labeled sciatic nerve with the provided electric stimulating device and observe the contracting response of gastrocnemius.

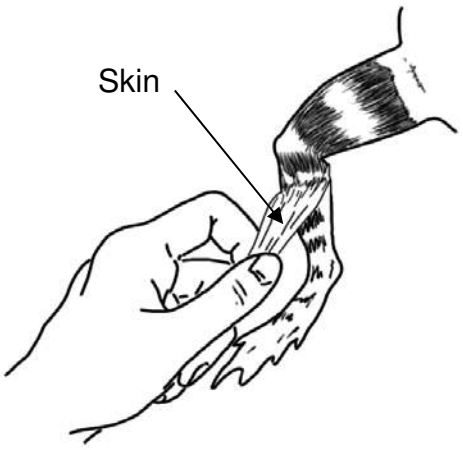


Fig. 4. Demonstration of skin peeling

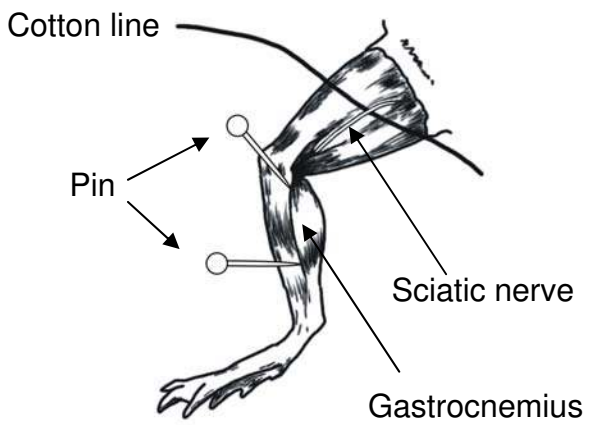


Fig. 5 Sciatic nerve and gastrocnemius

Q.1.2. (8 points) When you have finished the steps 6 to 10, **lift the sign** to notify the Lab assistant to videotape the contraction.

Step 11 to 12: (To keep the tissues wet, a small amount of Ringer's solution may be added onto the tissues any time)

11. Completely separate and isolate the intact sciatic nerve-gastrocnemius muscle preparation from the bullfrog specimen and place it in a petri dish, as shown in Fig. 6. (Sciatic nerve must be at least 2 cm long).
12. Stimulate the sciatic nerve with the electric stimulating device and observe the contracting response of the gastrocnemius.

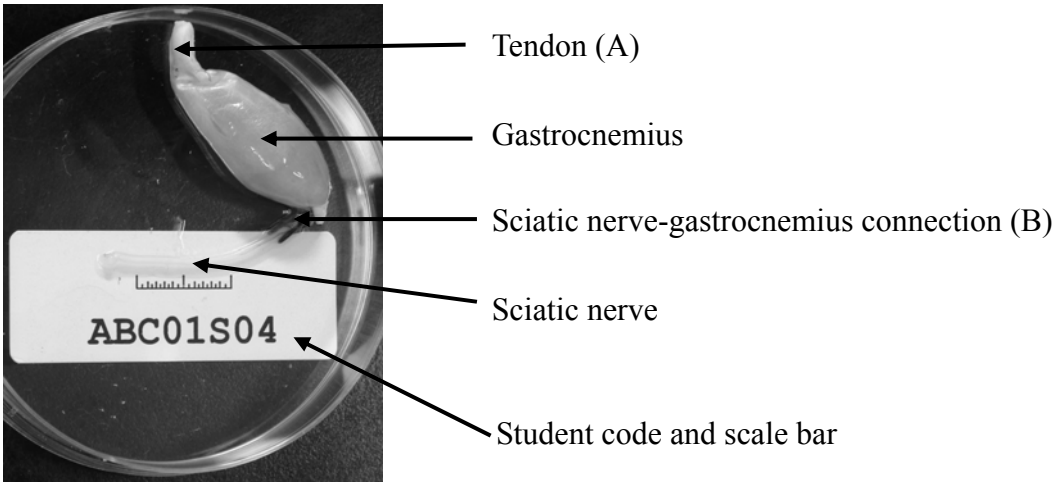


Fig. 6 Isolation of 「sciatic nerve-gastrocnemius」tissue

Q.1.3. (40 points) When you have finished steps 11-12, ~~write down the results of your observation on the answer sheets. Then,~~ **lift the sign** to notify the Lab assistant for checking the results and videotape the contraction.

Task II (43 points)

Identify tissues based on their morphology and match their functionality

Introduction:

The vertebrate physiological system is established by the functional coordination of 11 organ systems, which include the skin, skeletal, muscle, nervous, endocrine, cardiovascular, lymphatic, respiratory, digestive, urinary, and reproductive systems.

Identify the specimens on the slides (30 points)

Slides A to J are tissue sections from vertebrates. Identify the tissues or cell types, based on their characteristic features, using microscope.

1. Vein	2. Artery	3. Ganglion	4. Neuron	5. Blood（frog）
6. Blood（human）	7. Ovary	8. Testis	9. Lung	10. Skeletal muscle
11. Smooth muscle	12. Cardiac muscle	13. Kidney	14. Cartilage	15. Bone
16. Pancreas	17. Intestine	18. Gastric tissue	19. Skin	20. Rectum

Q.2.1. (30 points) Match each slide specimen (A to J) with its correct name from 20 different tissue/organ names listed in above table. (Note: only one correct answer for each specimen). Fill in the correct number in the answer sheets.

Identify the sliced tissue and match their correct functions (13 points)

Fig. 1-9 are the enlarged pictures of parts of different mammalian tissues. Based on their structural features, identify the tissue ~~determine the precise sources of them~~ and answer the questions below. The functions of 11 organs are listed in the following table. Each specific function is assigned an alphabetic letter (A to K).

Symbol	Functional description
A	Producing vitamin D_3
B	Producing erythropoietin
C	Producing urea
D	Producing Surfactant to reduce the surface tension
E	Regulating the homeostasis of the pH of body fluid
F	Helping the vein compression and promoting blood stream back to the heart
G	Digesting proteins
H	Producing secretin
I	Producing inhibin
J	Major organ for the storage of calcium and phosphate
K	Producing progesterone

Q.2.2. (13 points) Correctly write down the functional symbols, i.e. the alphabetic letters (A to K), on the answer sheets. Match the organ with their functionalities. **Note**: some organs may have more than one function (1 point will be deducted for each incorrect answer and minimum score will not be less than zero).

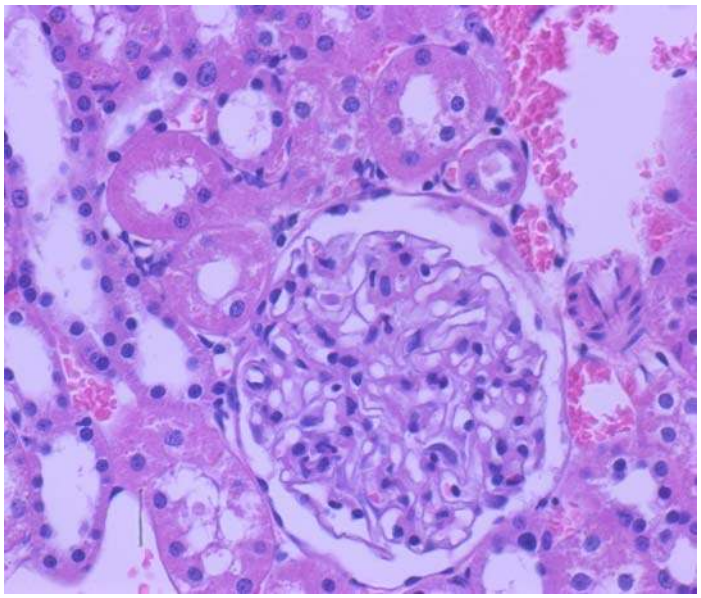
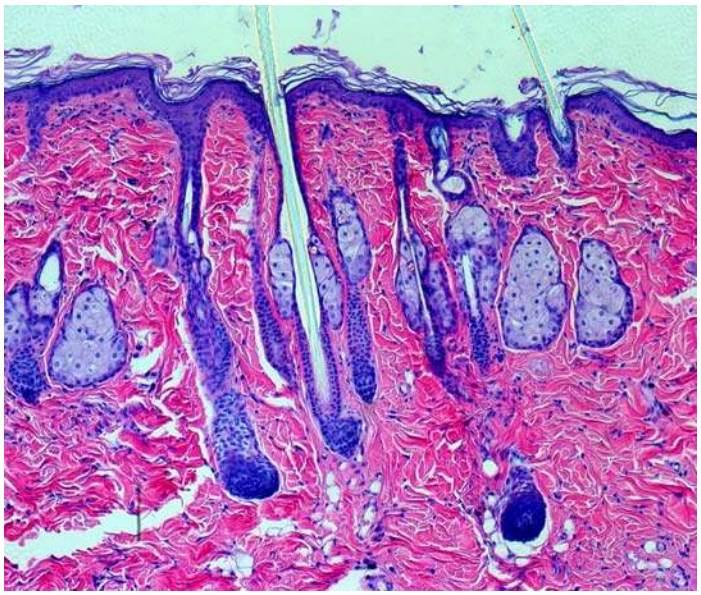
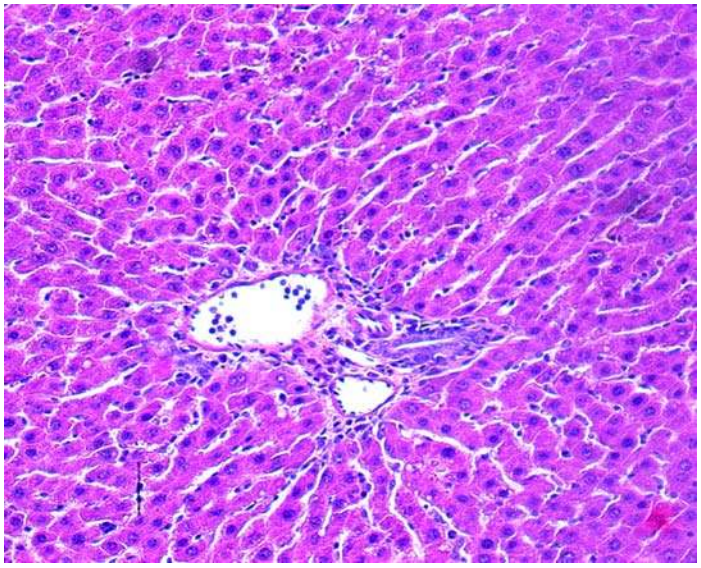
Fig 1.	 H&E stain (400 X)
Fig 2.	 H&E stain (100 X)
Fig 3.	 H&E stain (200 X)

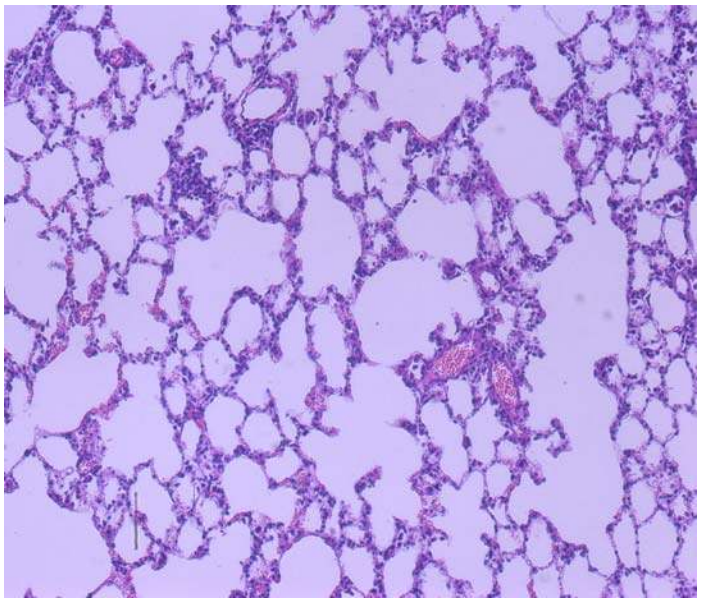
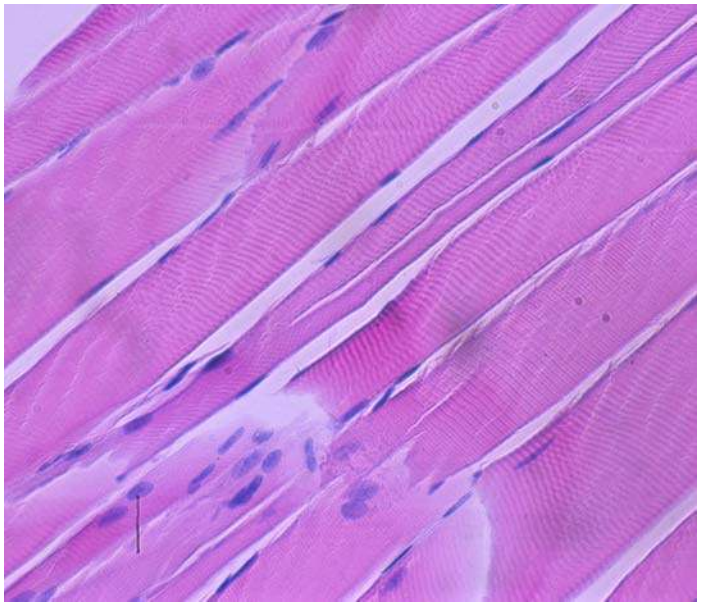
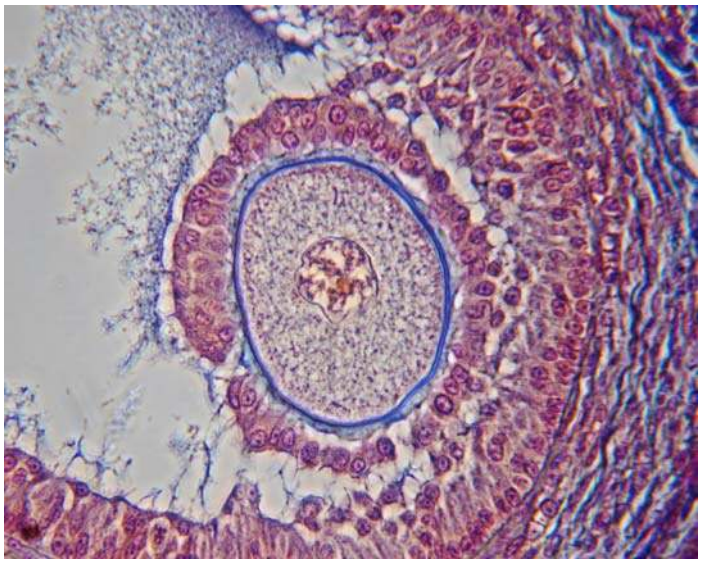
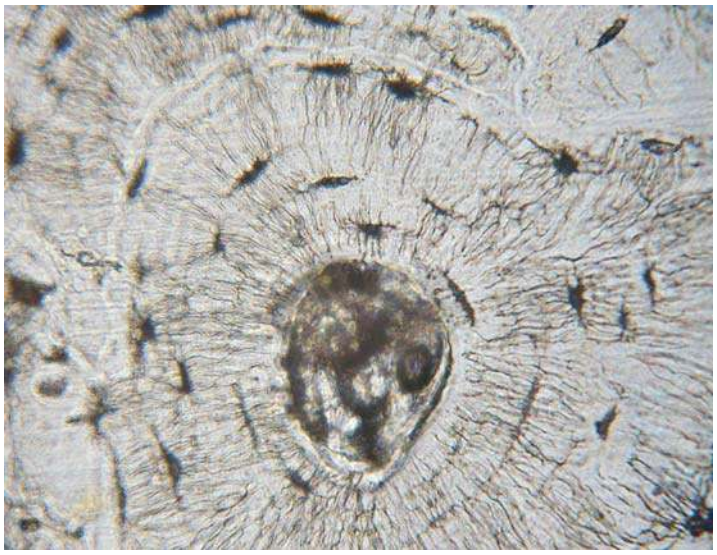
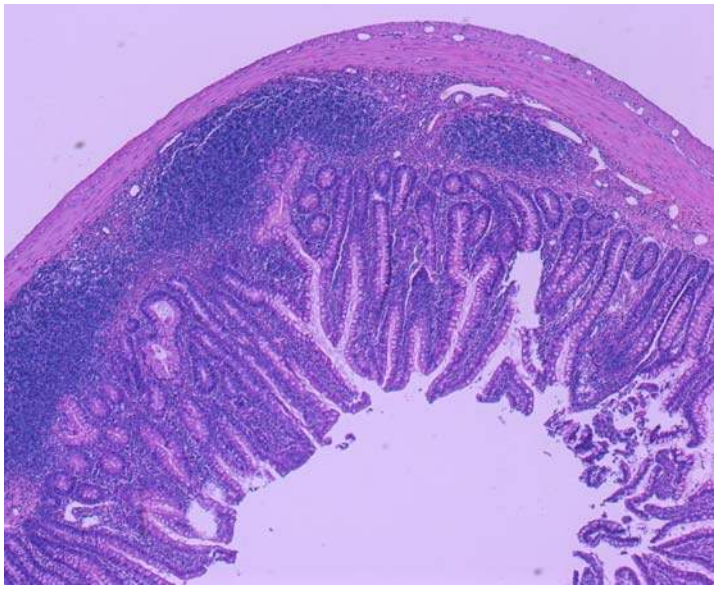
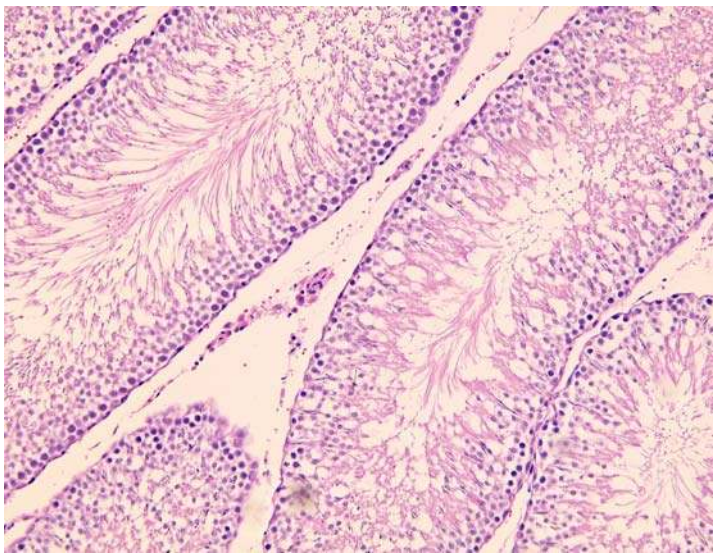
Fig 4.	 H&E stain (200 X)
Fig 5.	 H&E stain (200 X)
Fig 6.	 H&E stain (40 X)

Fig 7.	 (200 x)
Fig 8.	 H&E stain (100 X)
Fig 9.	 H&E stain (200 X)

PRACTICAL TEST 3 : ECOLOGY AND SYSTEMATICS

Total Points: 100 Duration: 90 minutes

Dear Participants,

- In this test, you have been given the following 2 tasks:

  Task I: Reconstruct the phylogenetic tree for the given spiders (60 points)

  Task II: Test of species association in a community (40 points)

- Check your **Student Code** on the **Answer Sheet** before starting the test.
- Write down your results and answers in the **Answer Sheet. Answers written in the Question Paper will not be evaluated.**
- Please make sure that you have received all the materials listed for each task. If any of the listed items is missing, please **raise your sign.**
- Use **pen only.** You can use a ruler and a calculator provided.
- **Check the condition of the spiders in the first 5 minutes.** If any of the legs is missing, please **raise your sign. No replacement of the spiders is possible after 5 minutes.**
- Stop answering and put down your pen **immediately** after the end bell rings.
- After test, our lab assistants will check the condition of the spiders and fill out the spider checklist at the end of your answer sheet. **Each undamaged spider in the original vial will get you one bonus point.** Please put down the **Student Code** after the check is done.
- Enclose both the **Answer Sheets** and **Question Paper** in the provided envelope after the spider check is finished. Our Lab Assistants will collect it promptly.

Good Luck!!

Equipments and Materials:

Equipments:

1	Dissecting microscope	1
2	Four sheets of colored pictures and one sheet of black and white picture: Figures (figure 1−3 to 1−12) Figure (figure 2-1)	 4 1
3	Forceps	2
4	Petri dish	2
5	70% ethanol	1
6	Plastic dropper	1
7	1-m quadrat cardboard (represented by a small cardboard in the zip lock bag)	1

Materials:

1	Four spider samples in glass vials (W, X, Y, Z)	1

TASK I: (60 points)

Reconstruct the phylogenetic tree for the given spiders

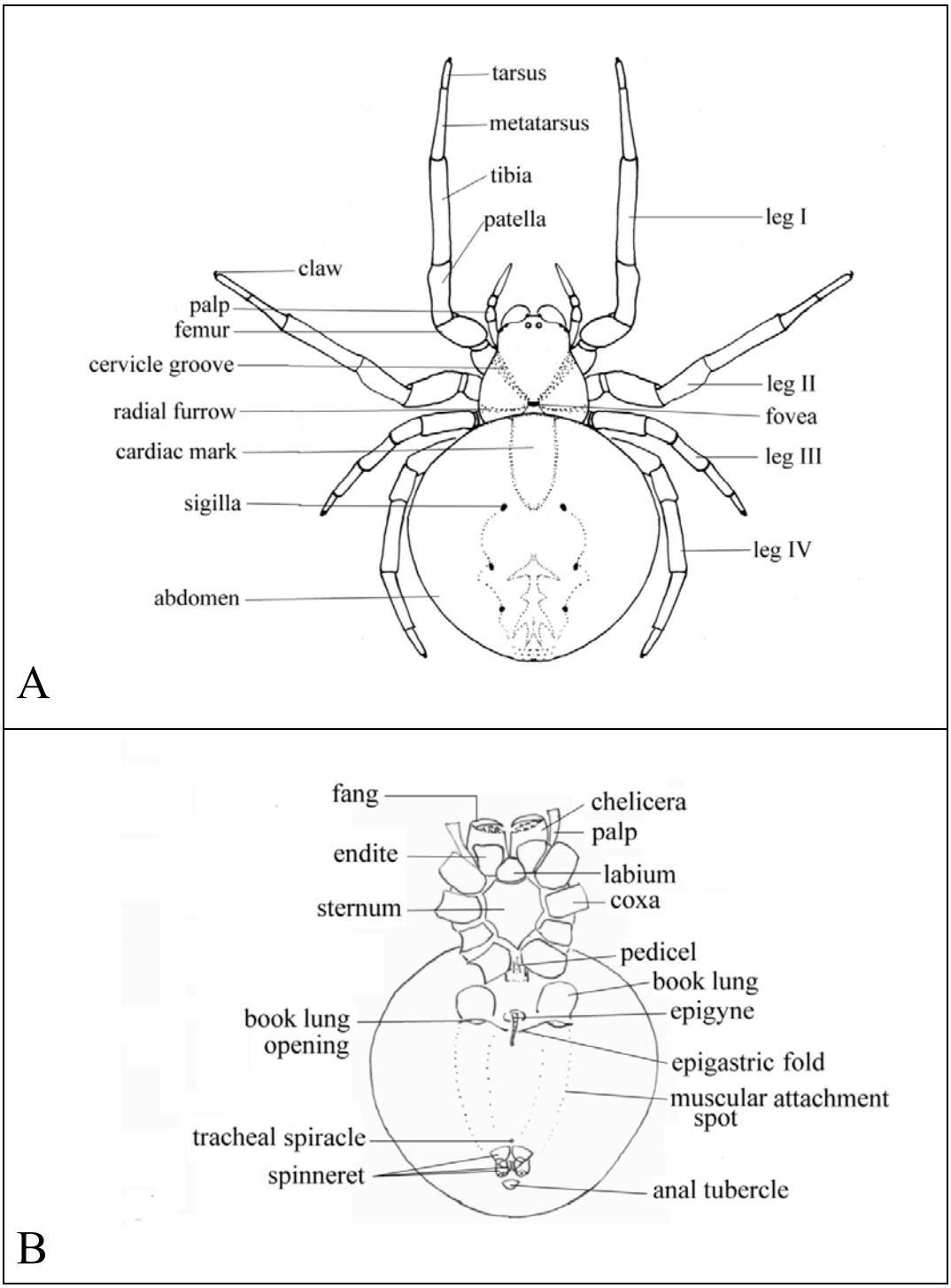


Figure 1-1 External morphology of spider. A. Dorsal view. B. Ventral view.

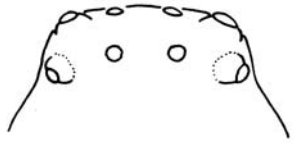
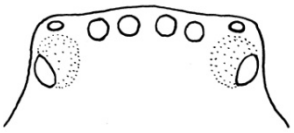
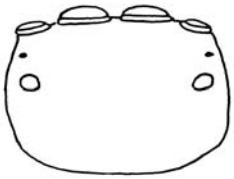
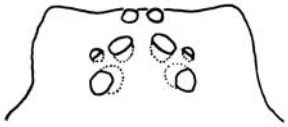
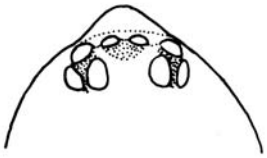
A: two transverse eye rows, with 4 eyes on each eye row 	B: two rows, with 6 eyes on the first eye row 	C: four rows, with 2 first row eyes smallest and 6 posterior eyes arranged in a hexagonal configuration 
D: three rows, with the 4 first row eyes much smaller than the others 	E: three rows, with the 2 anterior median eyes much larger than the others 	F: three rows, with 4 eyes on the second eye row 
G: eight eyes with one diad (a cluster of 2 eyes) and two triads (a cluster of 3 eyes) 	H: six eyes in two triads 	I: six eyes in three diads 

Figure 1-2 Eye arrangements (A key is provided on page 7-9)

Legends and Abbreviations of figures 1-3 to 1-12

Figure 1-3 Book lungs. A. Two pairs. B. One pair.

Figure 1-4 Spinnerets. A. Three pairs. B. Two pairs.

Figure 1-5 Cribellum. A. Absent. B. Present.

Figure 1-6 Calamistrum on metatarsus IV. A. Absent. B. Present.

Figure 1-7 Tarsi claw. A. Three claws. B. Two claws.

Figure 1-8 Claw tufts. A. Absent. B. Present.

Figure 1-9 Base of anterior spinnerets (AS). A. Widely separated. B. Close or in contact.

Figure 1-10 Grades of legs. A. Prograde. B. Laterigrade.

Figure 1-11 Tibia and metatarsus of legs I and II with series of long spines interspersed with much shorter setae. A. Absent. B. Present.

Figure 1-12 Double-rowed trichobothria on femora IV. A. Absent. B. Present.

1-1 Key to species of some common spiders

There are many living creatures in the world. For unfamiliar creatures, scientists usually choose a suitable key, the most commonly used tool, to find out its name. A key uses the dichotomous statements (a or b) of diagnosed characters to divide a larger group of taxa into two smaller subgroups (indicated by numbers or taxon names). Beginning with the number 1, choose a more likely statement (a or b) for the specimen and then go to the number shown at the end of the statement, and so on. Go through the key, until a taxon name is shown. A key for some common spiders of the world is given below.

Key to species of some common spiders

1a Two pairs of book lungs (Fig. 1-3A) ……2

1b One pair of book lungs (Fig. 1-3B) …… 3

2a Three pairs of spinnerets (Fig. 1-4A)……***A. aus***

2b Two pairs of spinnerets (Fig. 1-4B) ……***M. bus***

3a With a cribellum in front of the spinnerets (Fig. 1-5B), and a calamistrum on metatarsus IV (Fig. 1-6B) ……***Z. cus***

3b Without the cribellum and calamistrum (Figs. 1-5A, 1-6A) ……4

4a With six eyes ……5

4b With eight eyes ····· 6

5a six eyes in three diads (Fig. 1-2I) ····· ***S. dus***

5b six eyes in two triads (Fig. 1-2H) ····· ***P. eus***

6a Tarsi with two claws (Fig. 1-7B), with or without claw tufts ····· 7

6b Tarsi with three claws (Fig. 1-7A), never with claw tufts (Fig. 1-8A) ····· 10

7a Eyes in three or four rows (Figs. 1-2C, D, E, F) ·····8

7b Eyes in two rows (Figs. 1-2A, B) ····· 9

8a Eyes arranged in 4-2-2 three rows; with a pair of remarkably large anterior median eyes (AMEs) (Fig. 1-2E)·····***T. fus***

8b Eyes arranged in 2-4-2 three rows (Figs. 1-2F); AMEs not as above ····· ***C. gus***

9a Base of both anterior spinnerets separated from each other or wide apart (Fig. 1-9A); Legs prograde (Fig. 1-10A) ····· ***Z. hus***

9b Bases of both anterior spinnerets in contact (Fig. 1-9B); Legs laterigrade (Fig. 1-10B) ····· ***T. kus***

10a Eye group hexagonal, eyes arranged in 2-2-2-2 four rows (Fig. 1-2C)····· ***O. lus***

10b Eye group not hexagonal····· 11

11a Eyes in two rows (Figs. 1-2A, B)····· 12

11b Eyes in three rows (Figs. 1-2D, E, F)·····***P. mus***

12a Tibia and metatarsus of legs I and II armed with series of long spines interspersed with much shorter setae (Fig. 1-11B) ·····***M. nus***

12b Legs I and II without such spine arrangement····· 13

13a Femora IV with a proximal cluster of double-rowed trichobothria (Fig. 1-12B) ····· ***L. ous***

13b Femora IV without such trichobothria (Fig. 1-12A)····· ***N. pus***

Now, you have four spider specimens coded W to Z, respectively, as shown on outside of the vials. Please key out all these spiders and mark some of their characters with aid of figures 1-1 to 1-12. (**Caution!** You may take out the specimen from the vials for identification. When you do so, you should place a spider in the petridish with some 70% alcohol to examine its characters under the stereomicroscope. Because the spider's body is very fragile, the most ideal way to handle the specimen is gently grasping its legs with forceps to move it in or out from the vial. Don't break spider's body and its legs. Undamaged spiders in their original vials will get extra points as bonus. Please handle everything with care! Spiders should be kept in 70% alcohol at any time to prevent desiccation).

Q1.1.1 (4 points for each correct spider; 16 points total) Match each spider code with the correct taxon name respectively in your **Answer Sheet**.
Note: each spider code can only be used once, or the grades of these cells will not be counted.

Q1.1.2 (0.65 points for each cell; 13 points total) If a spider has the characters listed in the left column of the table in your **Answer Sheet**, indicate with a "＋" and if the character is absent, indicate with a "－". (Penalty of 0.2 points for each wrong answer, minimum 0 point)

1-2 Reconstruct a phylogenetic tree for eight spiders

Data matrix 1-1 represents character entries (a to t) for a group of hypothetical organisms A to H. Based on Data Matrix 1-1, Taxon A serves as the outgroup and the rest 7 species (Taxa B to H) are ingroups. Character state 0 represents the pleisiomorphy (ancestor character) and states 1-6 are apomorphies (derived characters). "-" represents missing character. We may reconstruct a cladogram (cladistic tree) by using the synapomorphies (shared derived characters). Each change represents one step of the evolutionary events (indicated by the character and its state, e.g., e-5, t-4). The following tree (Figure 1-13) is the only resulting most parsimonious cladogram that shows all the character changes on the tree. Numbers 1 to 15 represent 15 steps of the tree.

Data Matrix 1-1

Taxa	Character													
	a	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**m**	**n**	**o**	**p**	**s**	**t**
A	0	0	0	0	0	0	0	0	0	0	0	0	0	-
B	1	1	0	1	5	0	0	1	1	1	0	0	2	-
C	1	1	0	1	6	0	0	0	0	0	0	0	2	-
D	1	1	0	1	3	0	0	0	0	0	0	0	2	-
E	1	1	0	0	1	0	0	0	0	0	0	0	1	3
F	1	1	0	0	1	0	0	0	0	0	1	0	1	4
G	1	1	0	1	4	0	0	1	1	1	0	0	2	-
H	1	1	0	0	1	0	1	1	1	1	0	0	2	-

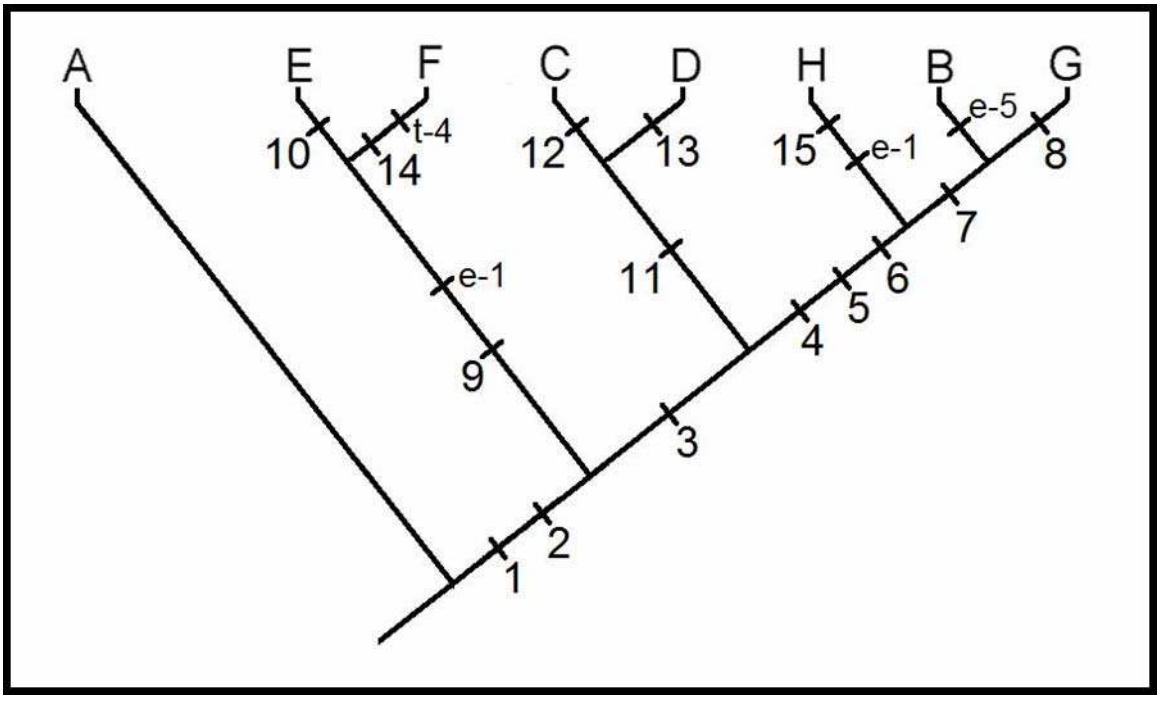


Figure 1-13 The most parsimonious cladogram reconstructed from data matrix 1-1.

Q1.2. (1.2 points for each cell; 18 points total) Fill in the character code and state (e.g., e-1) in the answer sheet for each of the 15 steps.

1-3 Based on the cladogram (figure 1-13), answer following questions:

Q1.3.1. (2 points) How many steps of the cladogram are there in total?

Q1.3.2. (2 points) Besides character e-1, which character is homoplasious (i.e., not homologous character)?

Q1.3.3. (2 points) Which of the following taxon is the sister group of taxon {C, D}?

(A) {E, F} (B) {H, B, G} (C) {F} (D) {H} (E) {B, G}

Q1.3.4. (Each correct answer will get 0.4 points, 2 points total) Mark with an "X" in the "True" cell in the **Answer Sheet** if the characters given below appeared prior to the evolution of character m-1 in the cladogram, otherwise in the "False" cell.

Character
s-1
s-2
a-1
g-1
d-1

Q1.3.5. (1 point for each cell; 5 points total) To what kind of grouping do the following taxa belong? Use code "I" for polyphyletic, "II" for paraphyletic, or "III" for monophyletic grouping.

Taxon
{H}
{B, C, G, H}
{C, D, E, F}
{B, G, H}
{B, E, G}

TASK II: (40 points)

Test of species association in a community

The basic idea of community organization is that species tend to be associated in a nonrandom manner. One way to understand their association conditions is to use a 2 × 2 contingency table (Table 2-1-0): If a sample contains both species x and y, it is defined as type "a". If a sample contains only species y, species x, or no species, then it is defined as type "b", "c", or "d" respectively.

Table 2-1-0

	Species x		
Species y	Present	Absent	Total
Present	a	b	a+b
Absent	c	d	c+d
Total	a+c	b+d	n

$n = a + b + c + d$

Probability of obtaining species x $P(x) = (a+c)/n$

Probability of obtaining species y $P(y) = (a+b)/n$

Joint probability (JP): the probability of both species x and y are present

$JP = P(x) \times P(y)$

Expected joint occurrences $= n \times JP$

Significance level for Chi-squared statistical test (χ^2)

Significance level (α) / df	0.05	0.01
1	3.841	6.635
2	5.991	9.210
3	7.815	11.345

Figure 2-1 (The figure on a separate paper on your table) is a distribution map of two plant species, Plant-A (○) and Plant-B (●), and a sympatric spider species, Spider (✻), in a hypothetical community. Each square is 0.5 × 0.5 m^2.

2-1 Association between Plant-A (○) and Spider (✻): analyzed by quadrat method.

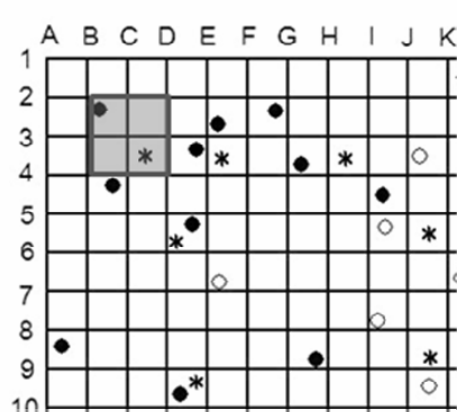
Put a 1-m square quadrat on Figure 2-1 using the following 40 randomly assigned coordinates as the center (i.e., 2 × 2 complete squares) and determine the type of each quadrat.

N-11, S-8, F-10, Q-18, O-16, K-2, L-4, M-17, M-4, H-17

X-2, K-11, T-19, M-8, P-10, G-8, B-19, M-19, S-10, O-12

J-18, D-7, B-17, I-11, B-10, G-13, V-16, C-3, F-5, R-15

L-2, Q-11, R-5, G-11, K-10, T-10, X-9, R-3, O-3, F-16



C-3 for example:

Q2.1.1. (1 point each; 9 points total) Write down your results in Table 2-1-1 and complete all the blank cells.

Answer the following in your Answer Sheet:

Q2.1.2a. (0.6 points) Calculate P (Plant-A).

Q2.1.2b. (0.6 points) Calculate P (Spider).

Q2.1.2c. (0.6 points) Calculate JP (Plant-A and Spider)

Q2.1.2d. (0.6 points) Calculate the expected joint occurrences.

Q2.1.2e. (0.6 points) Two species are more likely to be positively associated if the actual observation of the joint occurrence is greater than the expected one, and negatively associated if the actual observation is smaller than the expected one. According to the expected joint occurrence, what can be hypothesized about the kind of the association between Plant-A and Spider? [Answer Code: P for positive association, N for negative association.]

2-1-3 A simple Chi-squared statistical test (χ^2) with one degree of freedom (df = 1) is calculated as follows:

$$n = a + b + c + d$$

$$\chi^2 = \frac{n\,(ad-bc)^2}{(a+b)(c+d)(a+c)(b+d)}$$

Q2.1.3. (2 points) Based on Table 2-1-1, Calculate χ^2**. (to the fourth decimal place)**

2-1-4 The strength of the association between the two species can be estimated from a coefficient (V) defined as follows:

$$V = \frac{ad - bc}{\sqrt{(a+b)(c+d)(a+c)(b+d)}}$$

The V value varies from -1 (strongly negative association) to +1 (strongly positive association) and it is zero when there is no association.

Q2.1.4a. (2 points) Calculate the V value from Table 2-1-1. **(to the fourth decimal place)**

Q2.1.4b. (2 points) According to the V value, what can be hypothesized about the strength of the association between the two species? (Mark your answer with an "X" in the cell on the **Answer Sheet**)

2-2 The following table shows data using 40 randomly placed 2-m square quadrats.

Table 2-2-1

Plant-A (○)	Spider (∗)		Total
	Present	Absent	
Present	14	16	30
Absent	8	2	10
Total	22	18	40

The expected joint occurrence is 16.5.

The Chi-squared statistical test (χ^2) with one degree of freedom (df = 1) is calculated as $\chi^2 = 3.3670$.

V = -0.2901

Based on Table 2-2-1 answer following questions:

Q2.2.1a. (2 points) According to the expected joint occurrence, what can be hypothesized about the kind of the association between Plant-A and Spider? [Answer Code: P for positive association, N for negative association]

Q2.2.1b. (2 points) According to the V value, what can be hypothesized about the strength of the association between the two species? (Mark your answer with an "X" in the cell on the **Answer Sheet**)

2.2.2. (6 points total) Mark with an "X" on the **Answer Sheet** for each statement whether it is true or false.

Q2.2.2a. (2 points) Both tests of association using 1-m and 2-m square quadrats (sections 2-1 and 2-2) allowed us to reject the null hypothesis of random distribution.

Q2.2.2b. (2 points) The larger the quadrat size used, the more accurate the results.

Q2.2.2c. (2 points) Increasing the sampling efforts in the quadrat method should improve the accuracy of the results of species association.

2-3 Association between Plant-A (○) and Plant-B (●): analyzed by the nearest neighbor method.

Tally up the frequencies of the nearest neighbor of each plant systematically for all individuals. Fill in the totals in the table printed in the Answer Sheet.

Q2.3.1. (0.5 points for each cell; 3 points total) Write down your results in Table 2-3-1 and complete all the blank cells.

Q2.3.2a. (2 points) Based on Table 2-3-1 with one degree of freedom (df = 1), calculate χ^2.

(to the fourth decimal place)

Q2.3.2b. (3 points) Are these two plant species randomly distributed, associated or segregated? (Mark your answer with an "X" in the cell)

2-4 Mark with an "X" on the **Answer Sheet** for each statement whether it is true or false. **(4 points total)**

Q2.4.1. (2 points) The null hypothesis of the χ^2 test for the nearest neighbor method is that both Plant-A and Plant-B are randomly distributed.

Q2.4.2. (2 points) Using the nearest neighbor method to test species association can avoid the quadrat-size effect.

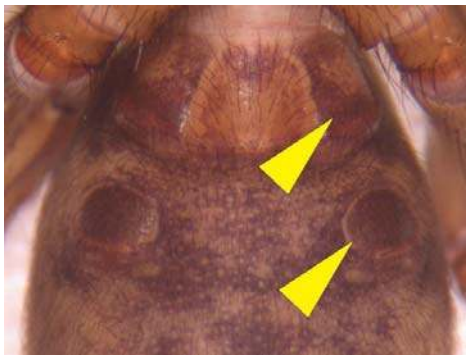
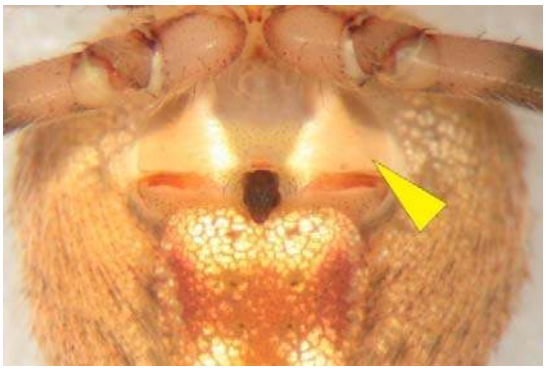
A: two pairs	B: one pair
	

Figure 1-3 Book lungs

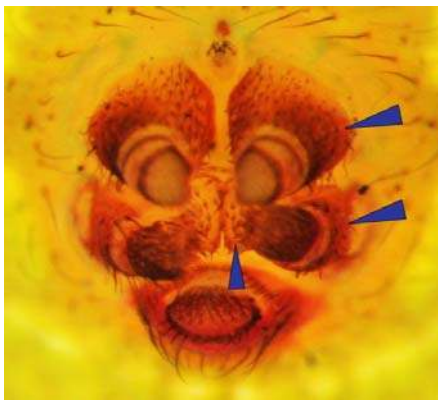
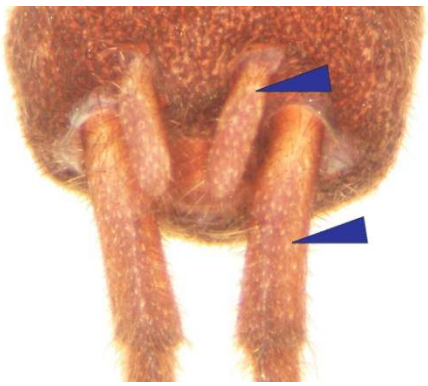
A: three pairs	B: two pairs
	

Figure 1-4 Spinnerets

A: absent	B: present
	

Figure 1-5 Cribellum

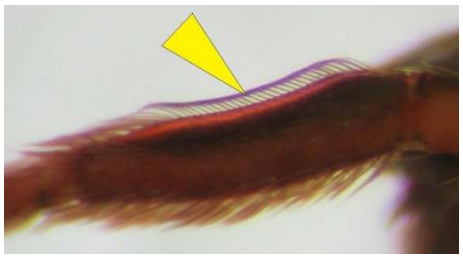
A: absent	B: present
	

Figure 1-6 Calamistrum on metatarsus IV

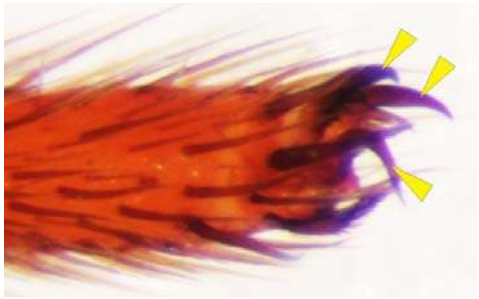
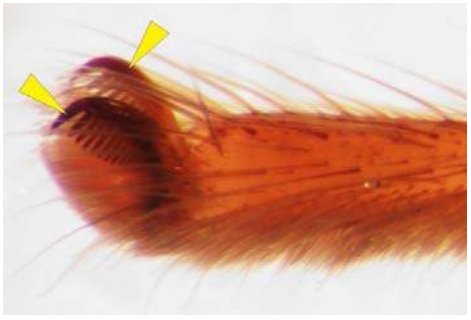
A: three claws	B: two claws
	

Figure 1-7 Tarsi claws

A: absent	B: present
	

Figure 1-8 Claw tufts

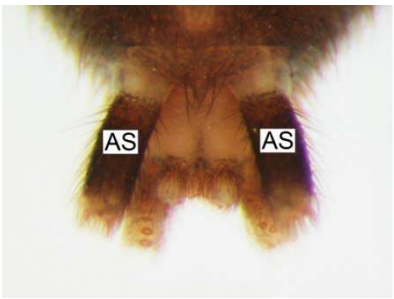
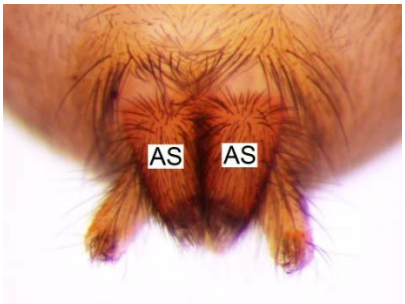
A: widely separated	B: close or in contact
	

Figure 1-9 Base of anterior spinnerets (AS)

A : prograde	B: laterigrade
	

Figure 1-10 Grades of Legs

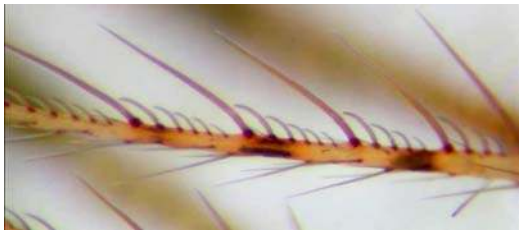
A: absent	B: present
	

Figure 1-11 Tibia and metatarsus of legs I and II with series of long spines interspersed with much shorter setae.

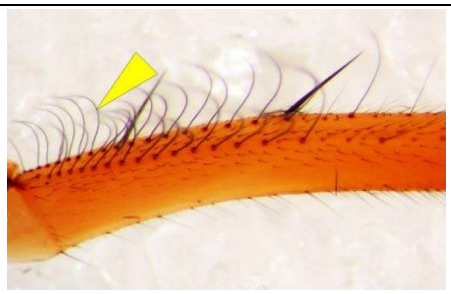
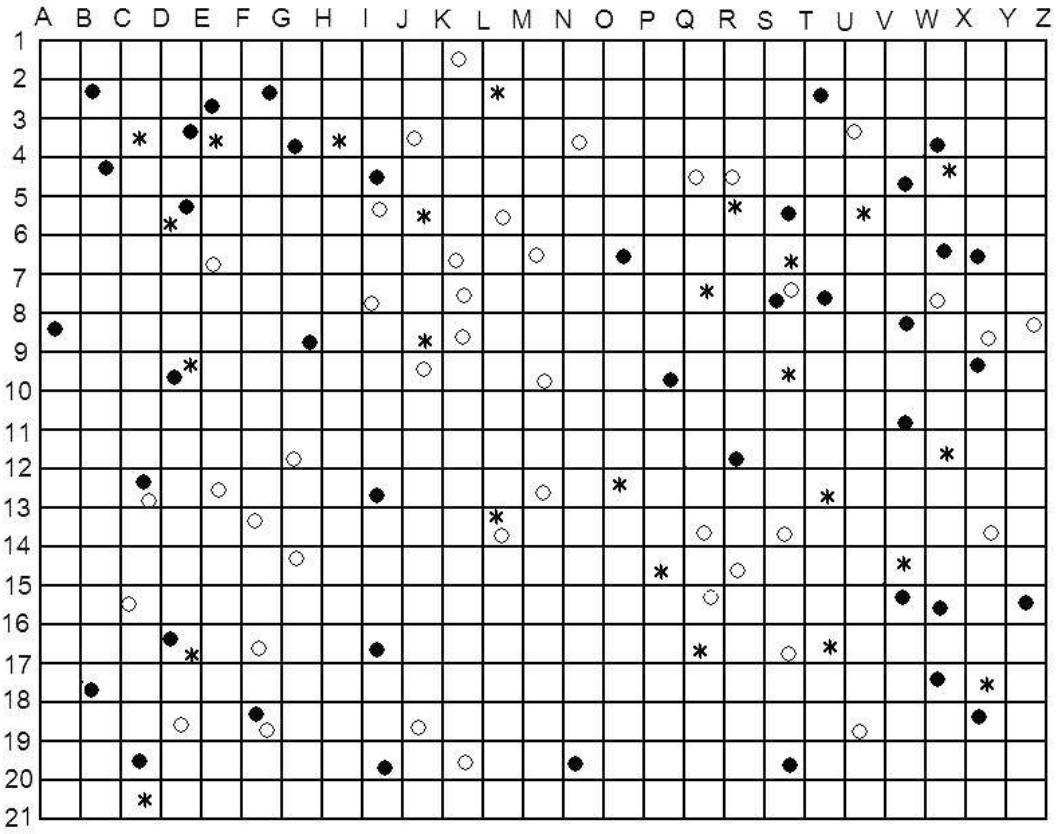
A: absent	B: present
	

Figure 1-12 Double-rowed trichobothria on femora IV


A B C D E F G H I J K L M N O P Q R S T U V W X Y Z
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

PRACTICAL TEST 4 : PLANT ANATOMY, PHYSIOLOGY, AND GENETICS

Total Points: 100 Duration: 90 minutes

Dear Participants,

- In this test, you have been given the following 2 tasks:

  Task I: Plant anatomy (**60 points**)

  Task II: Plant physiology and genetics (**40 points**)

- Check your **Student Code** on the **Answer Sheet** and **Template paper** before starting the test.
- Write down your results and answers in the **Answer Sheet**. **Answers written in the Question Paper will not be evaluated.**
- Make sure that you have received all the materials listed for each task. If any of the listed items is missing, **lift the sign.**
- Ensure that you organize the sequence of your tasks efficiently.
- Stop answering **immediately** after the end bell rings.
- After test, enclose the **Answer sheets**, **Question paper**, **Data printout**, and the **Template paper (without slides)** in the provided envelop. Our lab assistant will collect it promptly.
- No paper or materials should be taken out of the laboratory.

Good Luck!!

Task I (60 points)

Plant Anatomy

Equipments:	**Quantity**
1. Slides (in carrier box)	20
2. Cover slips (in carrier box)	30
3. Compound microscope (with 4X, 10X, and 40X objective lenses)	1
4. Ocular micrometer (installed within the lens)	1
5. Single sided razor blade (in carrier box)	5
6. Petri dish (in carrier box)	1
7. Forceps (in carrier box)	1
8. Marker pen	1
9. Kimwipes	1
10. Paper towel	1
11. Waste basket	1

Materials:	**Quantity**
1. Double distilled water (labeled as "ddH_2O" in carrier box)	20 mL/vial
2. 1 N hydrochloric acid (HCl) (in carrier box)	5-10 mL/vial
3. Transparent nail polish (in carrier box)	1 vial
4. Section slides X, Y, and Z of the root of plant K in slide box K	1 slide each
5. Four-compartment plastic petri dish (Containing tissue samples from plants V, W, M, N, P, Q, R, S in each compartment)	2 petri dishes
6. Template paper (with student code) for placing the slides with sections you made and for documenting	1 sheet

Part A: Structure of Plant Root (10 points total)

Introduction :

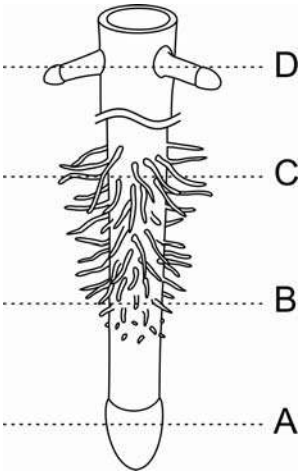


Figure 1 The structure of a typical plant root tip

There are three slides (X, Y, and Z) that are the transverse sections (located within the circular label on the slides) from different regions of the root of plant K. You need to determine whether plant K is monocot or dicot to answer questions later. Observe these sections under microscope and answer the following questions.

Q1.A.1.（2 point each, 6 points total）

Section X, Y, and Z each corresponds to which part of the root depicted in Figure 1. Check [X] the correct answers on the answer sheet.

Q.1.A.2. （single answer, 4 points）

What is the direction of maturation of primary xylem in the root (tissues mature earlier → tissues mature later)? Check [X] the correct answers on the answer sheet.

Part B: Structure of Plant Stem（6 points total）

Introduction :

Carefully prepare transverse sections of proper thickness from the stem segments of plants V and W and place them on separate slides. Add a drop of water onto the sections and cover with cover slips.

Observe under the microscope, and answer the following questions. You need to determine whether plants V and W are monocot or dicot to answer questions later. **When you finish this part, place your slides with the sections on the template paper, lift the sign and the lab assistant will mark with stamp in the boxes with slides.**

Q.1.B.（3 points for each plant, points are given when all correct answers are selected; 6 points total）

What are the distribution patterns of vascular bundles in the stems of plants V and W? Check [X] the correct answers on the answer sheet.

Part C: Structure of Plant Leaf（14 points total）

Introduction :

First determine the upper and lower epidermis of the leaves of plants M and N. Answer the following questions. You need to determine whether plants M and N are monocot or dicot to answer questions later. **When you finish this part, place your slides with the sections on the template paper, lift the sign and the lab assistant will mark with stamp in the boxes with slides.**

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Q.1.C.1（8 points）

Observation of stomata of plant M:

Prepare the upper and lower epidermis strips of the leaves, either by peeling them off with bare hands or by scraping off the undesired parts and leaving only the epidermis. Place these epidermal tissues on separate slides, with the epidermal side facing upward. Add a drop of water on each tissue sample and cover with cover slip. Observe under the microscope and use the ocular micrometer for measurements. One smallest unit of scale length of the micrometer is approximately 30 μm when observing under 4X objective lens. Answer the following questions in the answer sheet.

(a) Measurement of stomatal size on upper epidermis:

i) Under the 40X objective lens, what is the length of each smallest scale unit of the ocular micrometer? (1 point)

ii) Measure the lengths of 3 guard cells, then average their lengths. (3 points)

(b) Measurement of stomatal density on lower epidermis:

i) Under 40X objective lens, what is the approximate area of the field of view? (1 point)

ii) Observe 3 fields of view, and calculate the number of stomata in each. Then work out the average stomatal density. (3 points)

Q.1.C.2 （6 points, points are given when all correct answers are selected）

Observation of leaf tissue of plant N:

Use the provided transparent nail polish to paint the upper and lower epidermis of leaves. When the nail polish has dried, carefully peel off the layers and place on separate slides, with the epidermal side facing upward. Add a drop of water on each sample, cover with cover slips. Use the appropriate objective lens to observe. Determine the stomatal distribution of the upper and lower epidermis, and deduce the habitat of plant N. Check [X] the correct answers on the answer sheet.

Part D: Monocot or Dicot Plants (5 points)

Q.1.D (1 point each, 5 points total)

Determine whether plants K, V**,** W, M, and N are monocot or dicot. Check [X] the correct answers on the answer sheet.

Part E: Calcium Crystals in Plant Cells（20 points）

Introduction :

Some plants have idioblasts that can form polygonal calcium oxalate crystals or calcium carbonate crystals. Use the four plant materials (P, Q, R, S), carefully prepare transverse sections of appropriate thickness with a clean razor blade, and place the sections on separate slides. Add a drop of water on each section, and cover with cover slips. Observe under the microscope and check for the presence of crystals. If the crystals are present, locate the region of crystal distribution in the tissue (most crystals present in or absent from the cells of vascular bundles), carefully open the cover slip, remove the excess water around the sections, and add a few drops of HCl. Add cover slips again and observe the samples under the microscope and deduce the types of crystals that are present. **When you finish this part, place your slides with sections on the template paper, lift the sign and the lab assistant will mark with stamp in the boxes with slides.**

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Q.1.E （6 points for each plant with the presence of crystals, points are given when all correct answers are selected ; 2 points for the plant with the absence of crystals; 20 points total）

According to your observation, fill in the corresponding letters in the table in the answer sheet.

Plants: P, Q, R, S

Location of crystals: **A** (most crystals present in cells of vascular bundles)

B (crystals absent from the cells of vascular bundles)

Crystal type: **C** (polygonal calcium oxalate crystal) ; **D** (calcium carbonate crystal)

Documentation

When you finish all the parts (A to E) of Task I, double check whether you have collected all the stamps for this Task. If not, lift the sign and the lab assistant will check your template sheet. (0.5 point for each slide present, 5 points total).

Task II (40 points)

Plant Physiology and Genetics

Shared Equipment

ELISA reader

Equipments:

		Quantity
1.	Micropipettes P200 and P1000	1 each
2.	Micropipettes tips for P200 and P1000	1 box each
3.	96-well microplate	1
4.	1.5 mL microcentrifuge tubes (for preparation of standard solutions, use those labeled 0 μM, 25 μM 50 μM, 100 μM, 200 μM, 400 μM)	12 (6 extra unlabeled)
5.	80-well microcentrifuge tube rack / 4-way test tube rack	1 each
6.	Vortex mixer	1
7.	Marker pen	1

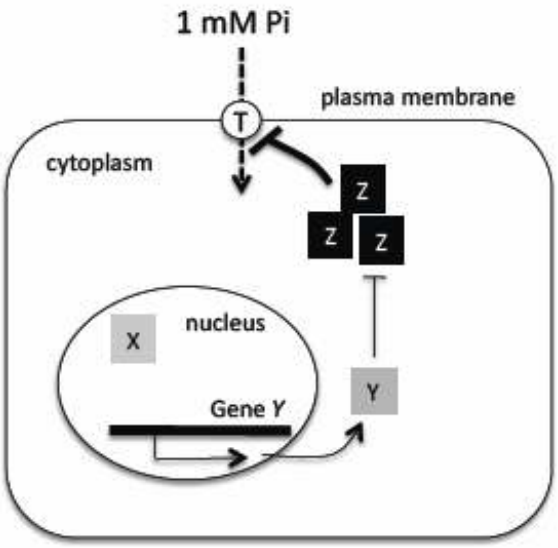
Materials:

		Quantity
1.	Phosphate detection solution (labeled as “Solution A”)	10 mL/tube
2.	400 μM KH_2PO_4 solution (labeled as “Solution B”)	10 mL/tube
3.	Double distilled water (labeled as “ddH_2O”)	50 mL/vial
4.	6 samples to be tested (allotted in microcentrifuge tubes, labeled as samples #1, #2, #3, #4, #5, & #6)	

Introduction:

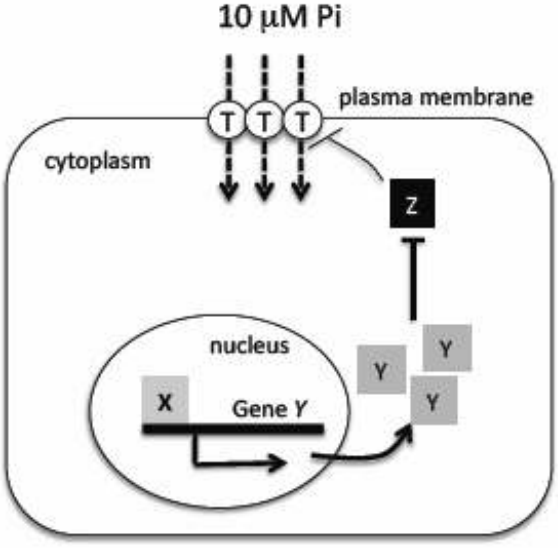
Phosphate is an important plant nutrient that constitutes substances such as cell membranes, nucleic acids, and energy compounds like ATP. When lacking phosphates, plants' growth and development can be dramatically affected. Plants can sense changes in phosphate concentration in the environment and accordingly regulate their gene expressions, changing the activity of phosphate transport proteins on the cell membranes to maintain the homeostasis of phosphate concentration within. Using the model plant *Arabidopsis*, scientists discovered that root cells respond to phosphate-sufficient (Pi-sufficient; e.g. 1mM) or phosphate-deficient (Pi-deficient; e.g. 10μM) condition as depicted below in Figures 2 and 3, respectively:

Figure 1



Pi-sufficient

Figure 2



Pi-deficient

When *Arabidopsis* is in Pi-sufficient condition (Figure 2), the protein Z negatively regulates the level of the protein T on plasma membrane, which is responsible for the transport of phosphate into the cell, to avoid excessive phosphate absorption that leads to toxicity. On the other hand, when plant is in Pi-deficient condition (Figure 3), the transcription factor X will enhance the expression of gene *Y* and thus increase the level of protein Y. Protein Y can promote the degradation of protein Z, leading to the increase of protein T level, and consequently a higher absorption of phosphate. In

general, the phosphate level in the shoot of a plant is proportional to the uptake efficiency of phosphate in the root.

The phosphate level in plants will be considerably affected when the expression of genes encoding T, X, Y, and Z is perturbed by mutation or transgene. Therefore, plant biologists can utilize such mutant or transgenic plants to determine the role and relationship of these genes in the regulatory mechanism of phosphate homeostasis.

There are 6 samples in microcentrifuge tubes, which are extracts from the shoots of five *Arabidopsis* lines (A to E) grown under either Pi-sufficient (1 mM) or Pi-deficient (10 μM) condition (as listed in Table 1). *Arabidopsis* A is wild type and B to E are either knockout (KO; null mutant with complete loss of function of the gene) mutant line or over-expression line corresponding to gene *T*, *X*, *Y*, or *Z*. You will be measuring the phosphate level in each sample and determine the identities of the samples based on the principles shown in Fig. 2 and 3. Each sample is derived from 20 seedlings of fresh weight shown in Table 1 and brought to final volume of 10 mL with ddH_2O .

Table 1

Sample No.	Plant	[Pi] in medium	Fresh weight of seedlings (mg)
1	A	1 mM	40.4
2	A	10 μM	17.3
3	B	1 mM	28.0
4	C	1 mM	39.2
5	D	1 mM	30.6
6	E	1 mM	33.8

Use the provided equipments and solutions to measure the phosphate concentration in each sample according to the experimental procedures

Experimental Procedures:

1. Use the 400 μM KH_2PO_4 solution (Solution B), ddH_2O, and pre-labeled 1.5 mL microcentrifuge tubes to prepare the following concentrations of phosphate solutions for standard curve: 0, 25, 50, 100, 200, 400 μM. Use vortex mixer to mix solutions thoroughly. For each concentration, there should be at least 0.5 mL. Use the P200 micropipette with fresh tips to transfer 0.1 mL of each standard solution into the 96-well microplate at specified positions (as in Figure 4, make 2 replicates for each standard).

Figure 4 Positions of standards and samples in 96-well microplate

			Standards			Samples			Standards				
A			0		#1	#1		0					
B			25		#2	#2		25					
C			50		#3	#3		50					
D			100		#4	#4		100					
E			200		#5	#5		200					
F			400		#6	#6		400					
G													
H													
	1	2	3	4	5	6	7	8	9	10	11	12	

2. Transfer 0.1 mL of each sample into the 96-well microplate at specified positions (as in Figure 4, make 2 replicates for each sample).
3. Add 0.1 mL of the phosphate detection solution (Solution A) into the wells that contain the standards and the samples. Mix by gently tapping the side of the plate.
4. **Lift the sign** after you finish Step 3, and wait for lab assistants to guide and help you with measuring the absorbance of the reaction mixtures with ELISA reader at 820 nm.
5. The lab assistant will print out the data for you. **Put your student code on the print-out**.
6. Answer the following questions:

Q.2.1. (18 points total)

Calculate the mean values of the absorbance for each sample and standard. Use the graph paper on the answer sheet to plot a standard curve. **(0.5 point for each standard point correctly plotted)**

Determine the phosphate concentrations of the samples in μM and the nmol phosphate per mg of seedling fresh weight for sample # 1 to # 6. Fill in your results in the table of answer sheet. **(2 points for each phosphate concentration measured, 0.5 point for each nmol/mg of phosphate calculated)**

Q.2.2. (Multiple answers, 4 points. Points given when all correct answers are selected)

For each of the following statements, determine whether they are true or false explanations for those plants having higher phosphate content (nmol/mg) than the wild type. Check [X] the correct answers on the answer sheet.

(A) X cannot be activated in the plant, thus leading to an increase in phosphate uptake.

(B) Loss-of- function of Gene *Y* in the plant causing an increase in phosphate uptake.

(C) Loss-of-function of Gene *Z* in the plant causing an increase in phosphate uptake.

(D) The plant harbors a transgene over-expressed gene *Y*, causing the loss of inhibition of protein T, leading to higher activity in phosphate uptake.

(E) Protein T of the plant has a defect, and it is unable to transport phosphate efficiently.

(F) The transcription factor X of the plant has a mutation, and is incapable to bind the promoter of gene *Y.*

Q.2.3. (2.5 points each, 10 points total)

According to the results from the experiment, assign the corresponding plant (B, C, D, or E) to the correct description on the answer sheet.

Q.2.4. (Single answer, 4 points)

If wild type plant (W), gene *X* knockout mutant (X), and gene *Y* knockout mutant (Y) were all grown in the same Pi-deficient condition, what would be the phosphate level in their shoots (rank from the lowest to highest)? Check [X] the correct answer on the answer sheet.

(A) X < W < Y

(B) Y < W < X

(C) W < X < Y

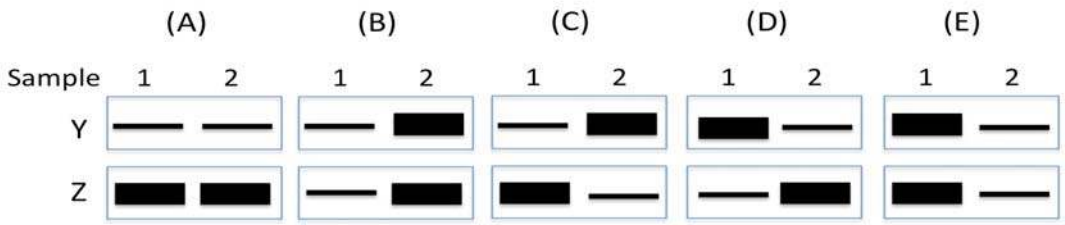
(D) W < Y < X

(E) X < Y < W

(F) Y < X < W

Q.2.5. (Single answer, 4 points)

Western blot is a technique to detect specific protein level with the use of antibody. Which of the following would be the most likely result of the Western blot analysis of protein Y and Z from the total protein extracts of samples # 1 and # 2? Check [X] the correct answer on the answer sheet.



Template paper for Task I (0.5 point for each slide present, 5 points total).

Part	Plant	
Part B	Plant V	Plant W
Part C	Plant M (upper epidermis)	Plant M (lower epidermis)
	Plant N (upper epidermis)	Plant N (lower epidermis)
Part E	Plant P	
	Plant Q	
	Plant R	
	Plant S	