Country Code: _____	Student Code: _____

The 21st INTERNATIONAL BIOLOGY OLYMPIAD

11th – 18th July, 2010

Changwon, KOREA

PRACTICAL TEST 1

PLANT AND ANIMAL SYSTEMATICS

Total Points: 50

Duration: 90 minutes

Dear Participants,

- In this test, you have been given the following 3 tasks:

  Task I: Reconstruct the phylogenetic tree of six plant species using parsimony method

  (25 points)

  Task II: Reconstruct the phylogenetic tree of six insect species using the UPGMA method

  (18 points)

  Task III: Co-evolution between plants and insects (7 points)

- 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 hand.

- Stop answering and put down your pencil **immediately** after the end bell rings. The supervisor will collect the Question Paper and the Answer Sheet.

Good Luck!!

PLANT AND ANIMAL SYSTEMATICS

This practical test is composed of 3 tasks.

TASK I. (25 points) Reconstruct the phylogenetic tree of six plant species using parsimony method

This task is composed of 4 parts.

Plant taxa (species)

A, B, C, D, E, and F.

Plant materials

Each set consists of the following materials:

1) The flowers of six species preserved in 70% ethanol (Flowers A-F).
2) The fruits of six species preserved in 70% ethanol (Fruits A-F).
3) Six dried flowering specimens (Flowering specimens A-F).
4) Six dried fruiting specimens (Fruiting specimens A-F).
5) Three prepared pollen slides (Each slide contains the pollen grains of two species, labeled A-B, C-D and E-F.).

Instruments

Stereomicroscope (20X), microscope (400X), razor blade, dissecting forceps, dissecting needles (2), petri-dishes (2), 20-cm ruler.

Part I-1. (9 points) Using the suggested materials and methods in Table 1, observe the following 10 characters. Enter each character state in the Data Matrix 1. Each character state should be recorded as the appropriate number (0, 1, or 2) based on the following descriptions. Figure 1 is provided as a reference of the descriptive terminology.

Table 1. Character descriptions for plants (See Figure 1 for illustration of the character states for the character 1, 2, 4, 7, 8, 9 and 10.)

No	Character	Character state	Materials	Methods
1.	Flower petal	0: Sympetalous 1: Polypetalous	Flowers	Naked eye
2.	Inflorescence	0: Corymb or umbel 1: Raceme 2: Axillary or terminal	Flowering specimens	Naked eye
3.	Fruit stalk	0: Longer than 1 cm 1: Shorter than 1 cm	Fruiting specimens	Naked eye
4.	Ovary position	0: Superior 1: Half-inferior 2: Inferior	Flowers	Dissect with razor blade, Stereomicro-scope (20X)
5.	**Habit**	**0: Shrub** **1: Tree**	**Given answer**	**Given answer**
6.	Trichomes on the fruit surface	0: Hairless 1: Densely haired	Fruiting specimens	Naked eye
7.	Fruit shape and size at maturity	0: Cylindrical or circular with emarginated tip (Dia.<1 cm) 1: Circular with pointed tip (Dia.< 1 cm) 2: Circular-elliptic with pointed tip (Dia.≥ 1 cm)	Fruits and Fruiting specimens	Naked eye
8.	Fruit types	0: Pome or capsule 1: Drupe	Fruits	*Dissect with razor blade, Naked eye
**9.	Pollen grains	0: Tetrad 1: Monad	Pollen slides	Microscope (400X)
10.	Leaf margin at maturity	0: Entire or undulate 1: Serrate or dentate	Fruiting specimens	Naked eye

*If the endocarp is hard, carefully remove exocarp and mesocarp to identify the fruit type.

** Search pollen grains within a red circle of each specimen.

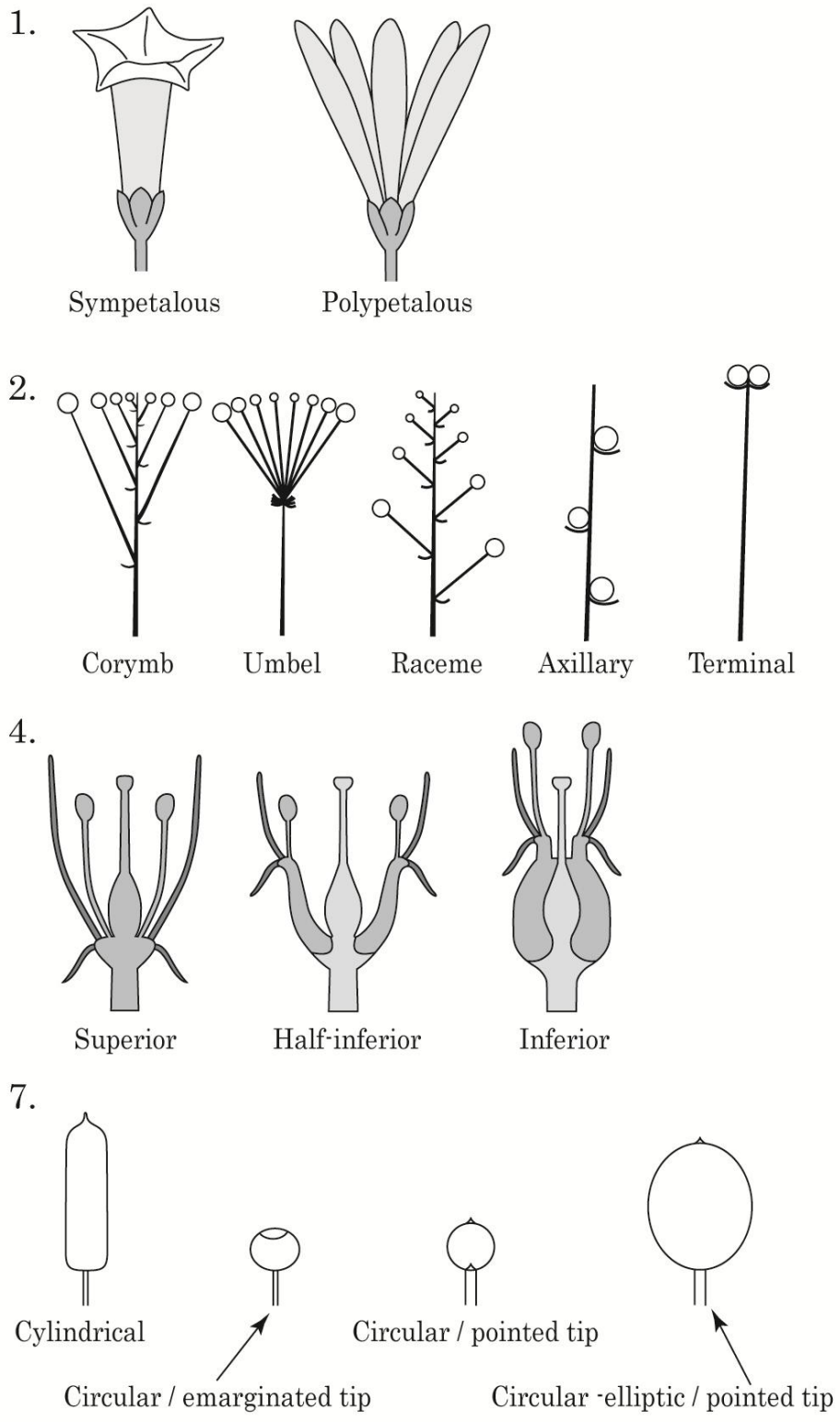


Figure 1. Illustration of character states for the character 1, 2, 4, 7, 8, 9 and 10.

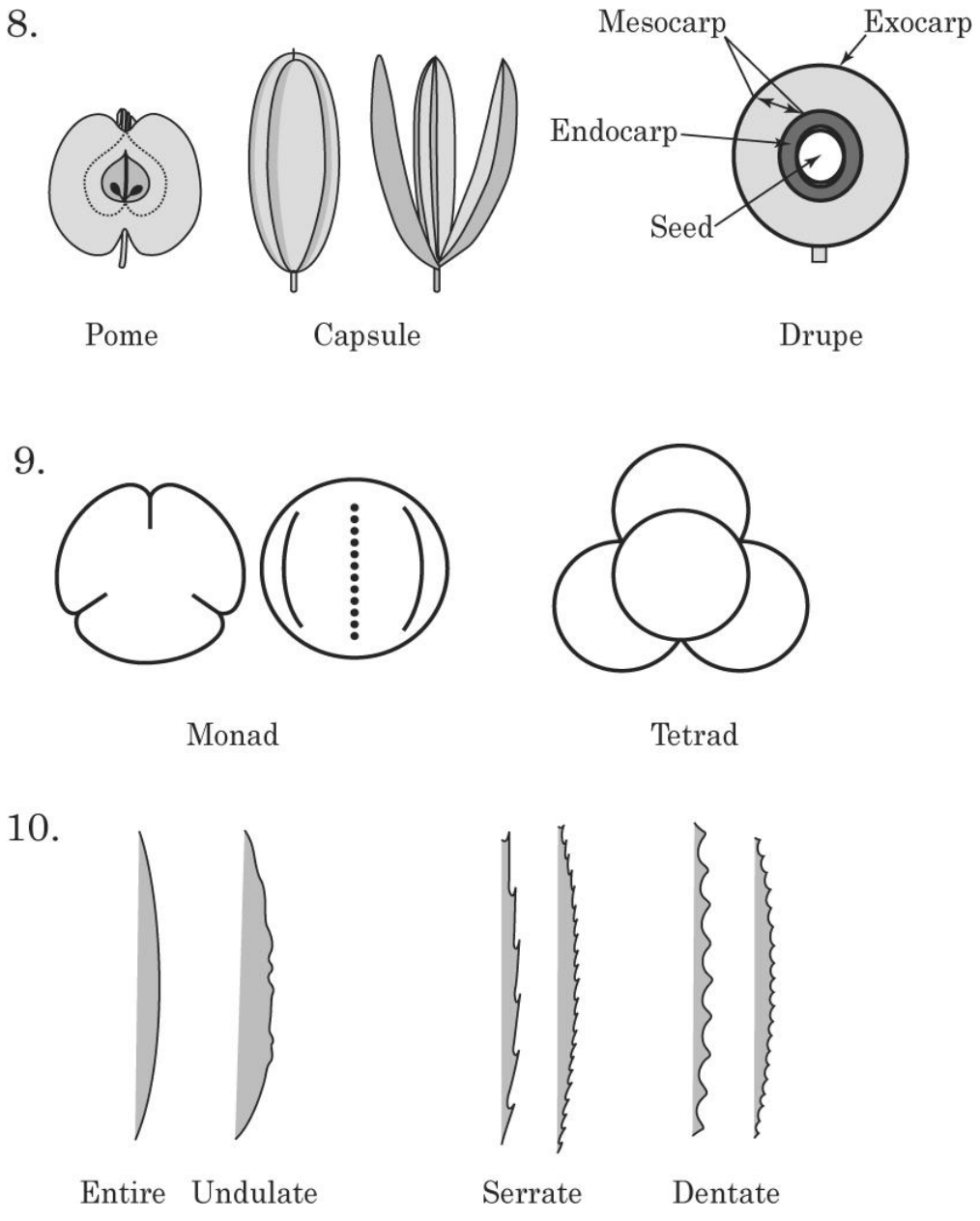


Figure 1. continued

Q1. (9 points) Fill the empty cells of Data Matrix 1 on your Answer Sheet. The sheet consists of 6 x 10 cells. Taxon F and character 5 are already filled for your reference.

Character / Taxa	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
A					**1**					
B					**1**					
C					**0**					
D					**0**					
E					**1**					
F	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Part I-2. (4 points) Refer to your completed Data Matrix 1 to answer the following questions

Q2.1. (1 point) Indicate with checkmarks (√) which of the characters are phylogenetically informative.

Q2.2. (1 point) Indicate with checkmarks (√) which of the characters are polymorphic (more than two states).

Q2.3. (2 points = 1 × 2) Use the following numerical formulae to define the number of possible trees for a given number of taxa (n).

The number of possible unrooted trees = $(2n-5)!/2^{n-3}(n-3)!$

The number of possible rooted trees = $(2n-3)!/2^{n-2}(n-2)!$

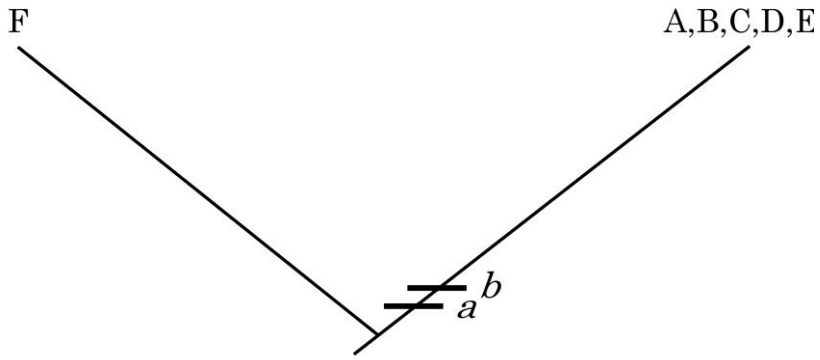
The symbol '**!**' in the formulae indicate the factorial.

What is the numbers of rooted and unrooted trees in this case?

Part I-3. (8 points) Cladistic analysis can be used to construct a phylogenetic tree of this species group. The primitive character state (plesiomorphy) is hypothesized to be the same as the state found in the outgroup F. Therefore, the character state 0 represents the primitive state for all given characters. Any change in state from that primitive trait is considered to be a derived character state, representing an evolutionary event (apomorphy). The character states of 1 and 2 represent derived condition. In this analysis, all characters are given equal weight. Tree construction is done in a step-by-step process. Place the appropriate character numbers and group members on the tree at each step.

Q3.1. (1 point) The initial Tree 1 can be created if we separate the ingroup (A, B, C, D, E) from the outgroup (F) using the two characters that distinguish all members of the ingroup (shared derived traits) from the outgroup F.

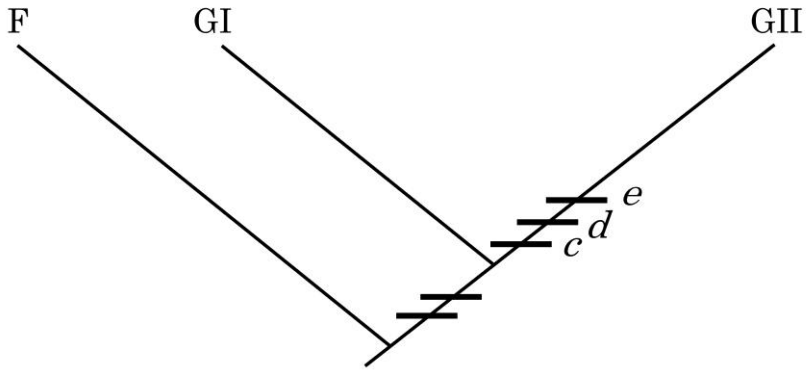
Identify these two characters (*a* and *b*) shown in Tree 1 and write them in the Answer Sheet. Character state should be given in parenthesis if the character is a polymorphic one.



Tree 1

Q3.2. (2 points) The step-by-step method to create the final tree from this initial tree can be illustrated by the concept of membership. Analysis proceeds to progressively less inclusive groupings supported by other derived characters. A less inclusive group(s) can be separated from the more inclusive group by the supported character change(s) on the tree. In the second step, the five ingroup taxa can be further divided into two subgroups (GI and GII) based on three synapomorphic characters.

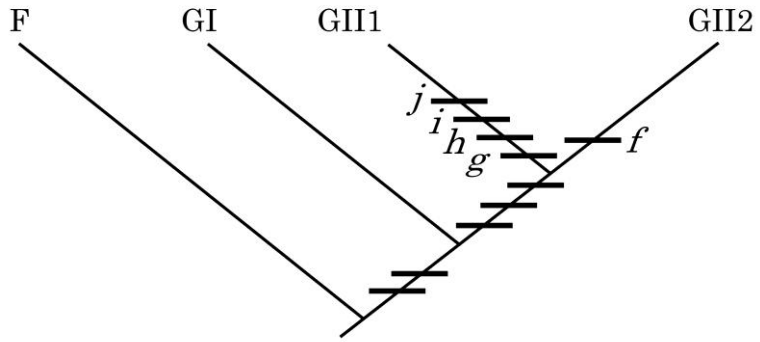
Identify the subdivided species group members of GI and GII and the three character numbers (*c*, *d* and *e*) shown on Tree 2 and write them in the Answer Sheet. Character state should be given in parenthesis if the character is a polymorphic one.



Tree 2

Q3.3. (3 points) The group II (GII) can be further divided into two less inclusive subgroups (GII1 and GII2) by four and one synapomorphic character(s), respectively.

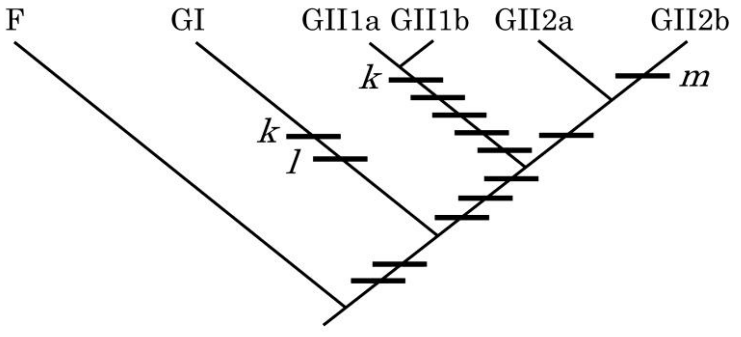
Identify the members of GII1 and GII2 and write the corresponding character numbers (shown in locations *f-j* in Tree 3) in the Answer Sheet. Character state should be given in parenthesis if the character is a polymorphic one.



Tree 3

Q3.4. (2 points) At the final stage of tree construction, all autapomorphic (singly derived) characters should be located on the tree, and any conflicting characters should be adjusted using the parsimony principle. There are two autapomorphic characters (*l* and *m*) and only a single conflicting character (*k*) in this case.

List the taxon name for each of the five ingroup species on the fully resolved Tree 4, and give the character numbers that correspond to *k*, *l* and *m*, respectively, in the Answer Sheet. Character state should be given in parenthesis if the character is a polymorphic one.



Tree 4

Part I-4. (4 points) Use the complete phylogenetic tree to answer the following questions.

Q4.1. (1 point) What is the number of character changes (steps) on the maximum parsimonious tree?

Q4.2. (1 point) The consistency index (CI) is defined as the minimum number of character state changes required in an absolutely consistent tree (all character states changed only once) divided by the observed number of character state changes in the final tree. What is the CI of the final Tree 4?

Q4.3. (1 point) What is the maximum number of genera that can be recognized from the final tree if taxa C and D are congeneric species?

Q4.4. (1 point) How many monophyletic groups can be recognized from the final tree?

TASK II. (18 points) Reconstruct the phylogenetic tree of six insect species using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) method

This task is composed of 3 parts.

Insect taxa: Six beetles (Coleoptera)

T1, T2, T3, T4, T5 and T6

Insect materials

Pinned and dried specimens of six beetles, labeled T1~T6.

Experiment tools

Insect stage, ruler, stereomicroscope (20X)

Note: Please be careful. Do not break the legs or antennae of the beetle specimens. There will be three point deduction penalty if you break the parts of any specimen.

Most insect specimens and their parts can be observed directly from the plastic case after removing the cover.

Part II-1. (8 points) The character states are defined in Table 2. Carefully observe the morphological characters of the beetle specimens using the naked eye and the stereomicroscope. Then, complete the Data Matrix 2. Figure 2 is provided as a reference for the Coleoptera body parts.

Table 2. Character descriptions for Coleoptera

	Character		Character state	Methods
1.	Longitudinal discontinuous ridges on elytra	0:	Present	Stereomicroscope
		1:	Absent	
2.	Horns on head and pronotum	0:	Absent	Naked eye
		1:	Present	
3.	Compound eye	0:	Does not surround antennal socket	Stereomicroscope
		1:	Surrounds about half of antennal socket	
4.	Mandible length	0:	Shorter than prothorax length	Naked eye, stereomicroscope if the part is small
		1:	Longer than prothorax length	
5.	Antennae length	0:	Shorter than body length	Naked eye
		1:	Longer than body length	
6.	Antennae shape	0:	Filiform or serrate	Naked eye, stereomicroscope if the part is small
		1:	Distal segments clubbed or lamellated	
7.	Antennae	0:	Not elbowed	Naked eye
		1:	Elbowed	
8.	Hind tarsi	0:	5 segments	Stereomicroscope
		1:	4 segments or less	
***9.**	**Notopleural sutures of the prothorax**	**0:**	**Fused**	**Given answer**
		1:	**Not fused**	
***10.**	**1st sternum and hind coxa**	**0:**	**Separated**	**Given answer**
		1:	**Not separated**	
***11.**	**Food preference**	**0:**	**Zoophagy or saprophagy**	**Given answer**
		1:	**Phytophagy**	

* Character states are provided in the Answer Sheet.

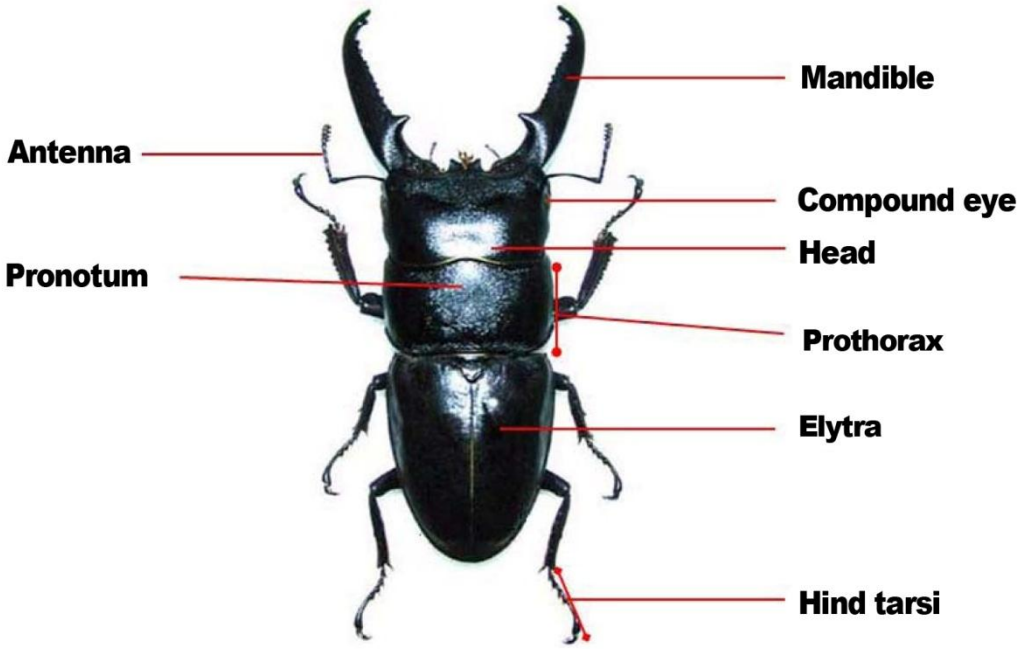


Figure 2. The body parts of Coleoptera to be observed.

Q5. (8 points) Complete the Data Matrix 2 in your answer sheet. The sheet consists of 6 x 11 cells. Characters 9, 10, and 11 are already filled in.

Data matrix 2.

Character / Taxa	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
T1									0	0	0
T2									1	1	0
T3									1	1	1
T4									1	1	1
T5									1	1	1
T6									1	1	1

Part II-2. (3 points) Create a character difference matrix between all possible pair-wise taxa from the completed Data Matrix 2. The difference value (D*ij*) between taxon *i* and *j* is the sum of the character (C) numbers for which $C_i \neq C_j$. Calculate the difference values, and fill the table below (Difference Matrix 1). The values of three pair-wise comparisons (T1/T2, T3/T4, and T5/T6) are already provided as examples.

Q6. (3 points) Complete the following Difference Matrix 1 on your answer sheet.

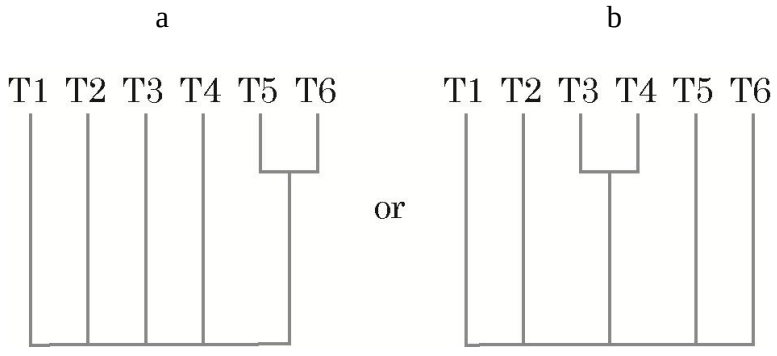
Difference Matrix 1. The pair-wise difference matrix calculation.

D*ij*	T1	T2	T3	T4	T5	T6
T1	-	-	-	-	-	-
T2	4	-	-	-	-	-
T3			-	-	-	-
T4			2	-	-	-
T5					-	-
T6					2	-

Part II-3. (7 points) Construct a phylogenetic tree based on the UPGMA algorithm using the pair-wise difference matrix that you created in Part 2. During the procedure, you will create nested clusters of taxa (smaller clusters into larger clusters) using successive difference matrices and phenograms until you construct a single cluster. The order for clustering is: 1) Pick the smallest entry D*ij*. 2) Join those two species into a cluster, 3) Compute new distances from that cluster to the other taxon *k*, using the Unweighted Pair Group Method with Arithmetic mean (UPGMA). A new distance between a new species *k* and a cluster (*i* and *j*) is defined as D(*k*(*ij*)) = (1/2)((D(*ki*)+D(*kj*)). Repeat the clustering process 1) ~ 3) to create the next cluster. This process should be continued to construct a single cluster for the entire group.

Q7.1. (1 point) Two alternate initial trees (a and b) can be constructed from the Difference Matrix 1 as shown below. Combine the two alternative trees and draw as a single tree (Tree 1). Draw Tree 1 on your Answer Sheet.

Initial Trees a and b: (T1, T2, T3, T4, (T5, T6)) or (T1, T2, (T3, T4), T5, T6)



Q7.2. (2 points) Complete the Difference Matrix 2. Calculate new difference values between cluster and taxon (or between cluster and cluster) using UPGMA algorithm and fill the cells in the answer sheet. Find the taxon pair(s) that shows the lowest difference values and make a newly clustered tree (Tree 2). Draw the tree in the answer sheet.

Difference Matrix 2:

D*ij* or D*k(ij)*	T1	T2	T(3,4)	T(5,6)
T1	-	-	-	-
T2	4	-	-	-
T(3,4)			-	-
T(5,6)				-

Q7.3. (2 points) Complete the Difference Matrix 3. Again calculate the new difference values between cluster and taxon (or between cluster and cluster) using UPGMA algorithm and fill the cells in the answer sheet. Find the taxon pair that shows the lowest difference values and make a newly clustered tree (Tree 3). Draw the tree in the answer sheet.

Difference Matrix 3:

D*ij* or D*k(ij)*			
	-	-	-
		-	-
			-

Q7.4. (2 points) Complete the Difference Matrix 4. Calculate the new difference values between cluster and cluster using UPGMA algorithm and fill the cells in the answer sheet. Make a complete clustered tree (Tree 4) and draw it in the answer sheet.

Difference Matrix 4:

D*ij* or D*k(ij)*		
	-	-
		-

TASK III. (7 points) Co-evolution between plants and insects

Plant-herbivore relationships are one of the core explanations for the rapid diversification of insects and flowering plants. For this task, use the final plant phylogeny (Task 1) and beetle phylogeny (Task 2). Under the assumption that the larvae of each insect species feed on a single plant species, compare the insect and plant phylogenies and answer the following questions.

Q8. (3 points) If the insect species T2, T3, and T5 feed on the plant species E, D, and A, respectively, what kinds of plant species are fed upon by insect species T1, T4, and T6, respectively?

Q9. (2 points) Which plant and insect species pair shows different phylogenetic positions in the trees?

Q10. (2 points) Which are the two best possible reasons to explain the differences between the insect and plant phylogenies? (Select the **two** best answers).

A. Host shift of insect species

B. Adaptive radiation of plant species

C. Genetic bottleneck during the insect species evolution

D. Different tree reconstruction methods

E. Genetic drift of plant species

Country Code: _____	Student Code: _____

PRACTICAL TEST 1

Answer Sheet

PLANT AND ANIMAL SYSTEMATICS

Total Points: 50

Duration: 90 minutes

TASK I. (25 points)

Part I-1. (9 points)

Q1. (9 points)

Data Matrix 1. (One point per character)

Character / Taxa	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
A					**1**					
B					**1**					
C					**0**					
D					**0**					
E					**1**					
F	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Part I-2. (4 points) Place "√" symbol on the right characters.

Q2.1. (1 point)

Character Number	1	2	3	4	5	6	7	8	9	10
Phylogenetically informative										

Q2.2. (1 point)

Character Number	1	2	3	4	5	6	7	8	9	10
Polymorphic										

Q2.3. (2 points = 1 x 2)

The number of possible unrooted trees: _____
The number of possible rooted trees: _____

Part I-3. (8 points) Character state should be given in the parenthesis if the character is polymorphic one.

Q3.1. (1 point)

	Character numbers
Character a and b	

Q3.2. (2 points). Character number and taxon name will be assign one point, respectively.

	Character numbers		Taxon name(s)
Character c, d and e		GI	
		GII	

Q3.3. (3 points)

	Character number(s)		Taxon name(s)
Character f		GII1	
Character g, h, i and j		GII2	

Q3.4. (2 points)

	Character number		Taxon name
Character *k*		GI	
Character *l*		GII1a	
Character *m*		GII1b	
		GII2a	
		GII2b	

Part I-4. (4 points)

Q4.1. (1 point)

steps

Q4.2. (1 point)

CI =

Q4.3 .(1 point)

Q4.4. (1 point)

Task II. (18 points)

Part II-1. (8 points)

Q5. (8 points)

Data Matrix 2

Character / Taxa	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
T1									**0**	**0**	**0**
T2									**1**	**1**	**0**
T3									**1**	**1**	**1**
T4									**1**	**1**	**1**
T5									**1**	**1**	**1**
T6									**1**	**1**	**1**

Part II-2. (3 points)

Q6. (3 points)

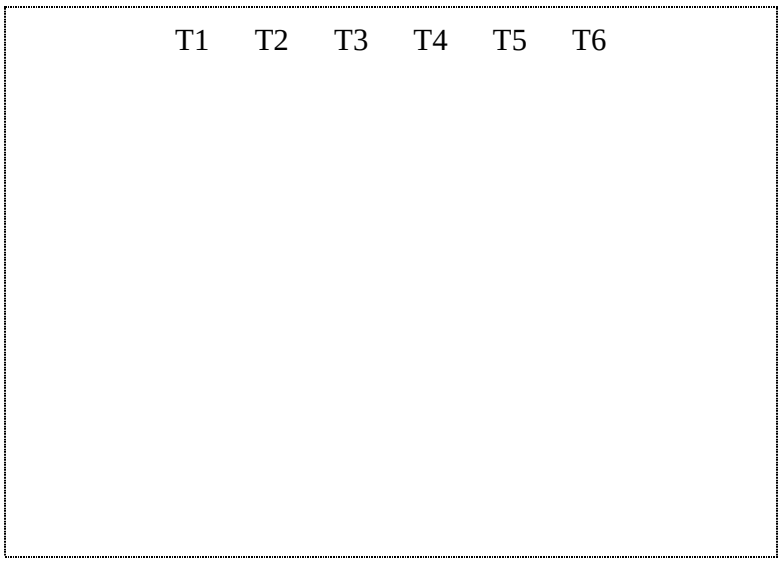
Difference Matrix 1

Dij	T1	T2	T3	T4	T5	T6
T1	-	-	-	-	-	-
T2	**4**	-	-	-	-	-
T3			-	-	-	-
T4			**2**	-	-	-
T5					-	-
T6					**2**	-

Part II-3 (7 points)

Q7.1. (1 point)

Tree 1: Combine and draw the two alternative trees as a single tree

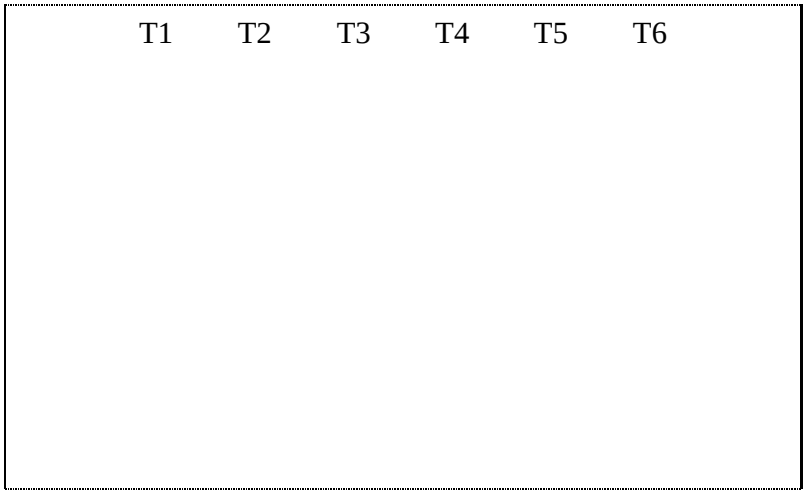


Q7.2. (2 points)

Difference Matrix 2

D*ij* or D*k(ij)*	T1	T2	T(3, 4)	T(5, 6)
T1	-	-	-	-
T2	**4**	-	-	-
T(3, 4)			-	-
T(5, 6)				-

Tree 2

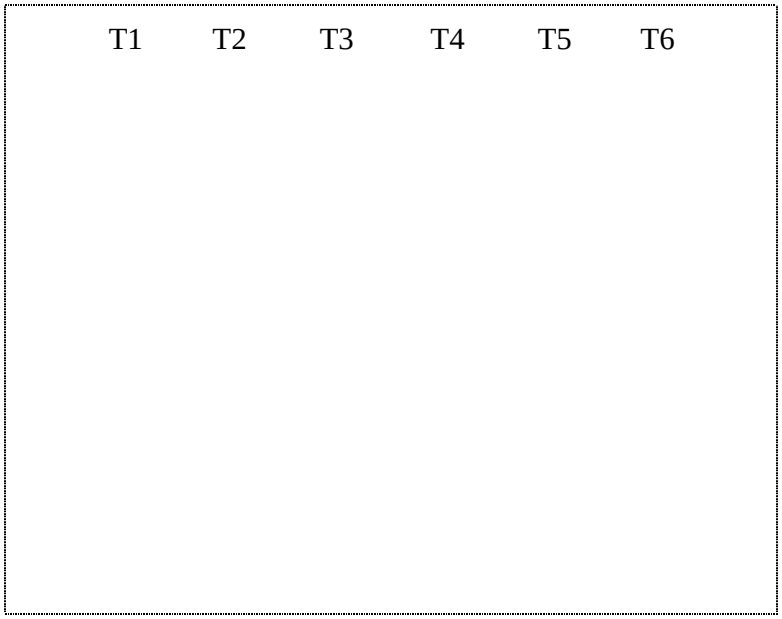


Q7.3. (2 points)

Difference Matrix 3:

D*ij* or D*k(ij)*			
	-	-	-
		-	-
			-

Tree 3

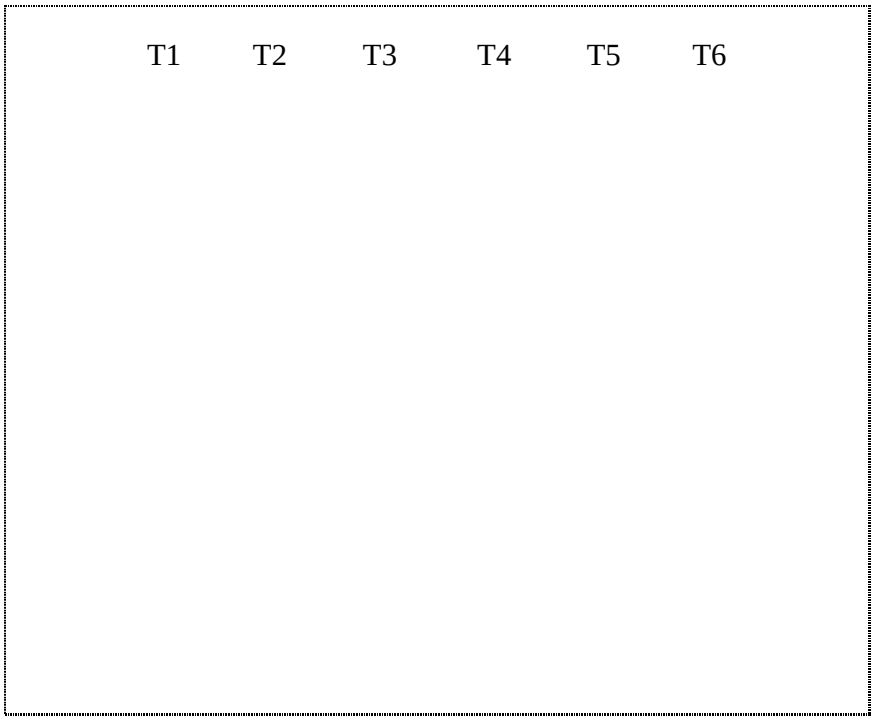


Q7.4. (2 points)

Difference Matrix 4

D*ij* or D*k(ij)*		
	-	-
		-

Tree 4



Task III. (7 points)

Q8. (3 points)

Insect Species	Plant Species
T1	
T2	**E**
T3	**D**
T4	
T5	**A**
T6	

Q9. (2 points)

Insect species	Plant species

Q10. (2 points)

A	B	C	D	E

PRACTICAL TEST 1

Answer Key

PLANT AND ANIMAL SYSTEMATICS

Total Points: 50

Duration: 90 minutes

TASK I. (25 points)

Part I-1. (9 points)

Q1. (9 points)

Data Matrix 1. **(**1 point per character) / (0.2 point/box)

Character / Taxa	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
A	1	2	1	1	**1**	1	2	1	1	1
B	1	2	1	1	**1**	1	2	1	1	1
C	1	0	0	1	**0**	0	1	1	1	1
D	1	1	0	1	**0**	0	1	1	1	1
E	1	0	0	2	**1**	0	0	0	1	0
F	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Part I-2. (4 points) Place "√" symbol on the right characters.

Q2.1. (1 point) (No partial score per character)

Character Number	1	2	3	4	5	6	7	8	9	10
Phylogenetically informative		√	√	√	√	√	√	√		√

Q2.2. (1 point) (No partial score)

Character Number	1	2	3	4	5	6	7	8	9	10
Polymorphic		√		√			√			

Q2.3. (2 points = 1 x 2) (1 point per box)

The number of possible unrooted trees: 105
The number of possible rooted trees: 945

Part I-3. (8 points) Character state should be given in the parenthesis if the character is polymorphic one.

Q3.1. (1 point) (0.5 point per character number)

	Character numbers
Character *a* and *b*	1, 9

Q3.2. (2 points)

	Character numbers		Taxon name(s)
Character *c*, *d* and *e*	4(1), 8, 10	GI	E
		GII	A, B, C, D

1. 1 point for Character numbers. (Deduct 0.3 point per wrong answer. Both character number and state should be correct for characters *c*,*d*, and *e*.)
2. 1 point for Taxon name(s) (0.5 point for GI, 0.5 point for GII)

Q3.3. (3 points)

	Character number(s)		Taxon name(s)
Character *f*	7(1)	GII1	A, B
Character *g*, *h*, *i* and *j*	2(2), 3, 6, 7(2)	GII2	C, D

1. 1 point for Character number(s) of character *f*. (no partial score.)
2. 1 point for Character number(s) of character *g*~*j*. (0.25 point per answer. Both character number and state should be correct.)
3. 1 point for Taxon name(s). (0.5 point for GII1, 0.5 point for GII2)

Q3.4. (2 points)

	Character number		Taxon name
Character *k*	5	GI	E
Character *l*	4(2)	GII1a	A
Character *m*	2(1)	GII1b	B
		GII2a	C
		GII2b	D

1. 1 point for Character number(s) of characters *k*~*m*. (Deduct 0.3 point per wrong answer. Both character number and state should be correct.)
2. 1 point for Taxon name. (0.2 point per box.)

Part I-4. (4 points)

Q4.1. (1 point)

14 **steps**

Q4.2. (1 point)

CI = 13/14

1. Any decimals possible. (0.92857……..)

Q4.3. (1 point)

4

Q4.4. (1 point)

5

Task II. (18 points)

Part II-1. (8 points)

Q5. (8 points)

Data Matrix 1

Character / Taxa	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
T1	0	0	0	0	0	0	0	0	**0**	**0**	**0**
T2	1	0	0	0	0	1	0	0	**1**	**1**	**0**
T3	1	0	0	1	0	1	1	0	**1**	**1**	**1**
T4	1	1	0	0	0	1	1	0	**1**	**1**	**1**
T5	1	0	1	0	1	0	0	1	**1**	**1**	**1**
T6	1	0	0	0	0	0	0	1	**1**	**1**	**1**

1. 1 point per each character. (Deduct 0.15 point per box with wrong number.)

Part II-2. (3 points)

Q6. (3 points = 0.25 × 12)

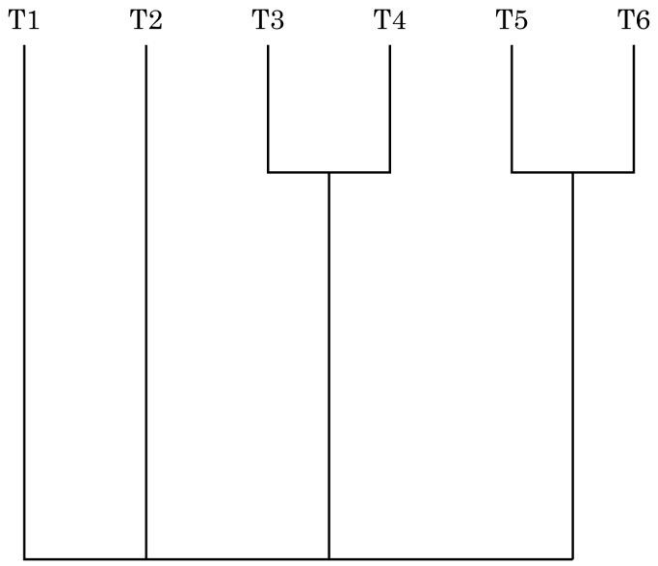
Difference Matrix 1

Dij	T1	T2	T3	T4	T5	T6
T1	-	-	-	-	-	-
T2	**4**	-	-	-	-	-
T3	7	3	-	-	-	-
T4	7	3	**2**	-	-	-
T5	7	5	6	6	-	-
T6	5	3	4	4	**2**	-

Part II.3 (7 points)

Q7.1. (1 point) (No partial score)

Tree 1: Combine and draw the two alternative trees as a single tree.

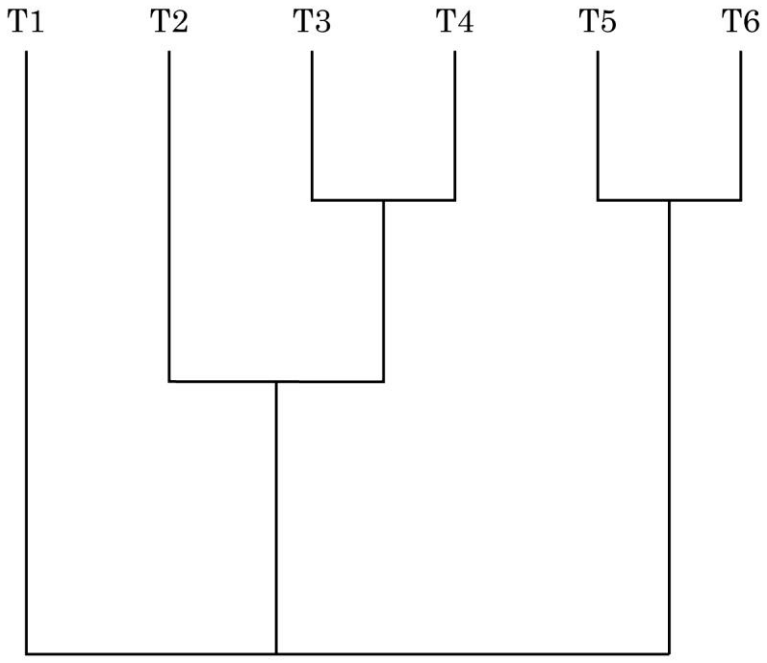


Q7.2. (2 points)

Difference Matrix 2 (1 point = 0.2 × 5)

D*ij* or D*k(ij)*	T1	T2	T(3,4)	T(5,6)
T1	-	-	-	-
T2	**4**	-	-	-
T(3,4)	7	3	-	-
T(5,6)	6	4	5	-

Tree 2 (1 point) (No partial score)

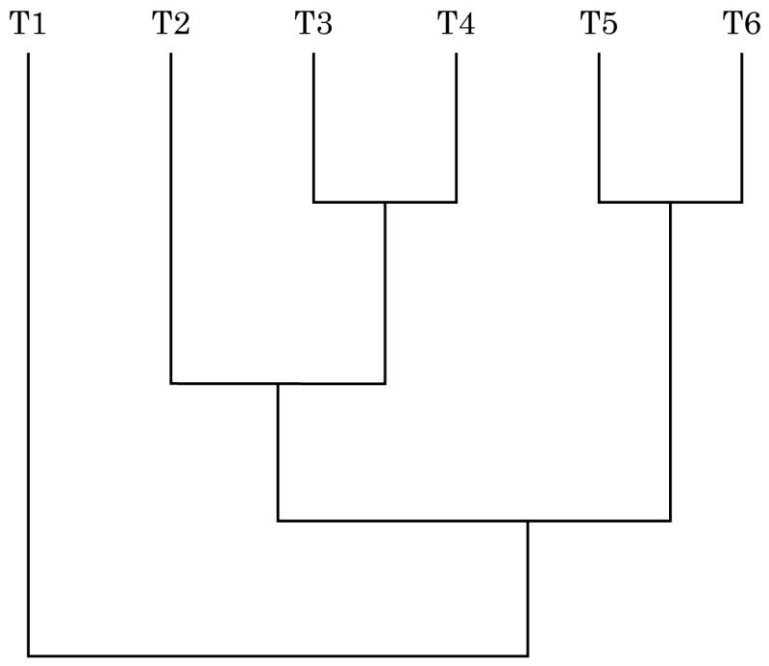


Q7.3. (2 points)

Difference Matrix 3 (1 point) (Deduct 0.1 point per box with wrong answer.)

D*ij* or D*k(ij)*	T1	T(2(3,4))	T(5,6)
T1	-	-	-
T(2(3,4))	5.5	-	-
T(5,6)	6	4.5	-

Tree 3 (1 point) (No partial score)

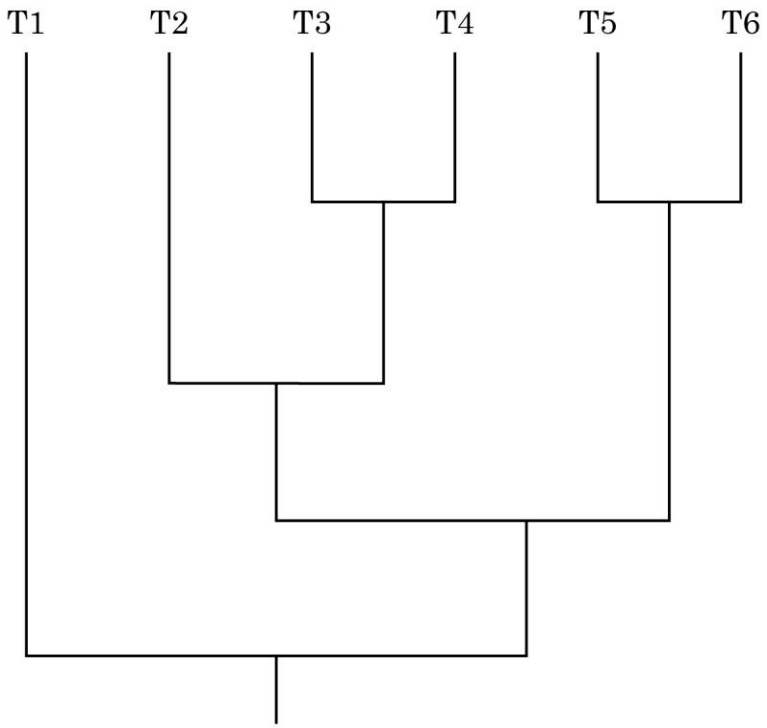


Q7.4. (2 points)

Difference Matrix 4 (1 point = 0.2 × 5)

D*ij* or D*k(ij)*	T1	T(2(3,4),(5,6))
T1	-	-
T(2(3,4),(5,6))	5.75	-

Tree 4 (1 point) (No partial score)



Task III. (7 points)

Q8. (3 points = 1 × 3)

Insect Species	Plant Species
T1	F
T2	**E**
T3	**D**
T4	C
T5	**A**
T6	B

Q9. (2 points = 1× 2)

Insect species	Plant species
T2	E

Q10. (2 points) (No partial score)

A	B	C	D	E
√			√	

Country Code: _____	Student Code: _____

The 21st INTERNATIONAL BIOLOGY OLYMPIAD

11th – 18th July, 2010

Changwon, KOREA

PRACTICAL TEST 2

PHYSIOLOGY AND ANATOMY

Total Points: 49

Duration: 90 minutes

Dear Participants,

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

  Task I: The response of the rat cerebral cortex to skin stimulation (25 points)

  Task II: Anatomy of spider (24 points)

- 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 hand.

- If you have any problem with your computer, raise your hand.

- Stop answering and put down your pencil **immediately** after the end bell rings. The supervisor will collect the Question Paper and the Answer Sheet.

Note: All animals used in the pictures and the described experiments were treated according to guidelines approved by the institutional animal care and use committee and conformed to the NIH guidelines on care and use of animals in research.

PHYSIOLOGY AND ANATOMY

This practical test is composed of 2 tasks.

TASK I. (25 points) The response of the rat cerebral cortex to skin stimulation

Welcome to the Electro-Physiology Laboratory!

Today you are going to examine one of the principles of how the brain works. This test is composed of 4 parts: one background section on how electrophysiological experiments are conducted and three experimental sections. You are required to answer a total of 15 questions by analyzing data presented on screen.

The home-page photo of the notebook computer shows the tools and equipment used in an electrophysiology laboratory.

Press 

above the photo

The primary somatosensory (S1) cortex receives tactile information from a specific body surface region. These specialized receptive areas in the human brain is shown in **Figure 1.** A similar body representation within the rat S1 (**Fig. 2**) will be created from these experiments.

Press  **or** 

1. Background information

1.1 Skull immobilization with brain exposure

The stereotaxic device is used to immobilize the skull (**Fig. 3**). The incisor bar is adjusted to make the skull surface horizontal (**Fig. 4**). Following a scalp incision, a hole is drilled in the skull over the location of S1, and a recording electrode (*a red, moving needle*) is inserted into the brain (**Fig. 5**). A micro-driver is used to move the electrode downward (25 μm/step) from the surface into the brain (**Fig. 6**).

Press  **or** 

1.2 S1 neuronal response following skin stimulation

The rat skin can be stimulated either mechanically with a cotton probe or electrically with an electrode. Following the electrical stimulation of forepaw digit (**Fig. 7;** *a white, moving arrow*), S1 neuronal activity is recorded using an electrode (**Fig. 7;** *a red, moving needle*). Using an oscilloscope (**Fig. 8**), S1 neuronal activity can be visualized (**Fig. 9**).

Press  **or** 

1.3 Response histogram

When an S1 neuron is responsive to the stimulation of a body part, the body part is within the receptive field (RF) of the neuron; a neuron does not show any response to the stimulation of body parts outside of its specified RF.

Using the amplifier (**Fig. 10**) and the analyzer (**Fig. 11**), activities of many S1 neurons surrounding the electrode can be recorded (**Fig. 12;** *left panel*). Subsequently, single neuronal activities can be isolated (**Fig. 12;** *spikes on the right panel*). To quantify the S1 neuronal responses, the stimulation of the body part is repeated within a certain period of time, and the

action potentials are accumulated to produce a histogram (**Fig. 13**). In the histogram, the X-axis stands for time (ms) before (-), the exact moment of (0), and after (+) stimulation. The Y-axis represents the mean firing rate (Hz) within the recorded neuron.

Press  **or** 

2. S1 neuronal response to forepaw digit stimulation

2.1. Forepaw digit region in S1

For the location of recording electrode, x-y coordinates are drawn over the skull (**Fig. 14**). The point where three bones meet (the bregma) is the origin (0, 0) of the coordinate system. Previous investigations reported that the point (0.3, 4.3) (**Fig. 15**) is one of the responding spots for stimulation of the **2nd** forepaw digit (**Fig. 16**).

Press  **or** 

2.2. Mechanical stimulation

To find the general boundaries of the S1 region responding to stimulation of a specific skin area, it is better to perform mechanical stimulation prior to electrical stimulation. A recording electrode is positioned above the coordinate (0.3, 4.3) and is lowered stepwise at **25 µm/step** (**Fig. 17;** *a red, moving needle*). The responses to mechanical stimulation of the **2nd** forepaw digit are given in Table 2.2

Table 2.2

Fig.	Depth (number of steps)	Area of skin stimulation	S1 response to skin stimulation	S1 Response to joint movement
18	0-30	Broad	Weak	no
19	31-48	Tip	Strong	no
20	49-60	Broad	Weak	strong

Press  **or** 

2.3 Electrical stimulation

A stimulating electrode is inserted into the **2nd** forepaw digit, whereas the recording electrode is inserted into the S1. The measured distance from the stimulating electrode to the recording one is ***12 cm***. The response of the S1 neuron to weak and strong stimuli is shown in Table 2.3. and Figures 21 and 22. (*Note the pop-up histogram at the bottom in both actions.*).

Table 2.3

Action	Stimulus to **2nd** forepaw digit	Response of S1 neuron
Cursor on 21	Weak (0.1 mA)	No conspicuous spike
Cursor on 22	Strong (2 mA)	One conspicuous spike

Q1. (1 point) Based on the results of mechanical and electrical stimulation, which of the following statements is correct?

A. The strongest response to mechanical stimulation is observed in neurons at 0.5-0.75 mm deep from the surface.

B. Neurons at a depth of 0.775-1.2 mm respond to the smallest skin area.

C. Neurons at a depth of 0.775-1.5 mm respond only to skin touch.

D. The thickness of the S1 cortex is less than 1mm.

E. The firing rate (Hz) of S1 neurons has no correlation with stimulus intensity.

Q2. (1 point) Calculate the minimum (p) and maximum (q) velocities (**unit: m/sec**) of information transmission from the digit to the S1.

Q3. (1 point) During the period of 6-15 ms after stimulation, what is the **net increase** in the mean value ($\overline{\mathbf{X}}$) of firing rate (Hz) evoked by strong (2 mA) stimulation?

2.4. Response to a gamma-aminobutyric acid (GABA) antagonist

GABA is a neurotransmitter in the brain. The response of the S1 neuron to weak and strong stimuli following the topical application of a GABA antagonist (i.e., inhibitor of GABA action) to the S1 cortex is shown in Table 2.4 and Figures 23 and 24. (*Note the pop-up histogram at the bottom in both cases.*).

Table 2.4

Action	Stimulus to **2nd** forepaw	Response of S1 neuron
Cursor on 23	Weak (0.1 mA)	No conspicuous spike
Cursor on 24	Strong (2 mA)	Two conspicuous spikes

Q4. (2 points) Based on the results of before and after the antagonist application, which of the following statements is correct?

A. The **net increase** in the mean firing rate (Hz) of the first peak in histogram **24** is about 2.14 times of that of the peak in histogram **22**.

B. After the antagonist application, the mean firing rates (Hz) always increase regardless of stimulation intensity.

C. The GABA antagonist inhibits excitatory synaptic activity in the S1.

D. Based on histogram **24**, a **net increase** in the mean firing rate (Hz) for the first peak is 4.5 times of the one for the second peak.

E. The second peak in histogram **24** is not associated with S1 processing of the cutaneous input from the digit.

Press  **or** 

3. S1 neuronal response to hindpaw digit stimulation

3.1 Electrical stimulation

Previous investigations reported that the point (-1.0, 2.5) is one of the responding spots for hindpaw digit stimulation (**Fig. 25**).

A recording electrode is lowered stepwise (**25 μm/step**) downward from the brain surface. Responses of neurons at three locations (**a=25 steps, b=41 steps, c=52 steps**) along the vertical track are recorded (**Fig. 26**).

Following strong (2mA) electrical stimulation of the **2nd**, **3rd**, and **4th** hindpaw digits (**Fig. 27**), responses of the three neurons at **a**, **b**, and **c** are recorded (**Fig. 29**).

3.2 Response to local anesthesia

A local anesthetic drug applied to the **3rd** hindpaw digit (**Fig. 28**, *grey color*) causes a sensory loss within 2 minutes, and the effect lasts for 30 minutes. Afterward, recovery of sensation gradually occurs. The drug effect completely disappears by 60 minutes post-application. When strong (2 mA) electrical stimulation is applied to the digit 40 minutes after drug application, the response of the three neurons is changed (**Fig. 30**).

Q5. (1 point) Based on neural response before anesthesia (**Fig. 29**), choose the correct statement.

Case	Neurons	Stronger or longer response	Weaker or shorter response
A	Locations **a**, **b** and **c**	**2nd** digit	**3rd** digit
B	Locations **a**, **b** and **c**	**4th** digit	**3rd** digit
C	Location **b**	**4th** digit	**2nd** digit
D	During **3rd** digit stimulation, neurons at locations **a** and **c** have longer response durations than neuron at location **b**.		
E	Location **a**	**4th** digit	Other digits

Q6. (1 point) Based on neural response before anesthesia (**Fig. 29**), choose the correct statement.

A. All three neurons respond to **4th** digit stimulation.

B. A single S1 neuron responds to the stimulation of only one digit.

C. Neurons at location **a** respond to the stimulation of more of the hindpaw digits than neurons at location **b**.

D. Neurons at location **c** respond to the stimulation of more of the hindpaw digits than neurons at location **b**.

E. All three neurons receive convergent sensory information from two or more digits.

Q7. (1 point) Based on the responses shown by the neurons in all three locations in **Figs. 29** and **30,** choose the **incorrect** statement.

Case	Location of stimulation	Timing of response	Magnitude of response
A	**2nd** digit	40 min after drug application	Increased
B	**3rd** digit	40 min after drug application	Decreased
C	**4th** digit	40 min after drug application	Increased
D	**2nd** and **4th** digits	Before and after drug application	Greater in **4th** than in **2nd** digit
E	A neuron not responding to a certain stimulus may respond to it under certain conditions		

Q8. (2 points) Based on the response after anesthesia (**Fig. 30**), select an appropriate inference.

A. The drug is absorbed into the blood and is transferred to the S1.

B. The drug has changed the structure of peripheral nerve branches.

C. Neuronal response is not altered after local anesthesia.

D. The drug causes reversible, temporary changes in S1 neuronal synapses.

E. The change in response after anesthesia is due to newly-synthesized proteins within the S1.

Press  **or** 

4. S1 body map

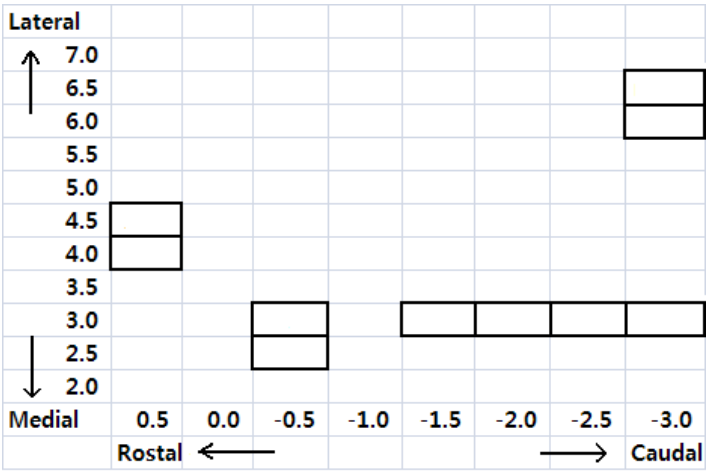
4.1 Normal S1 map

Following repeated stimulation/recording procedures, the normal S1 body map (**Fig. 31**) is obtained (**Note**: the electrode is moved along the x or y axis by the distance of 0.5 mm). If the computer cursor is laid on each symbol (○, ●, ▲, □), the *abbreviation* for appropriate body surface is shown as a note and, at the same time, the equivalent body position will be depicted at the bottom. The following table provides the anatomical term for each abbreviation used in the figure.

	Abbreviations	
forelimb	**fl**	forelimb
	fp	forepaw
	fpd 1-5	forepaw digits 1-5
	fm	forelimb muscle
hindlimb	**hl**	hindlimb
	hp	hindpaw
	hpd 1-5	hindpaw digits 1-5
	hm	hindlimb muscle
trunk	**t**	trunk
vibrissa	**mv**	mystacial vibrissa
	rv	rostral vibrissa

Q9. (5 points = 0.5 × 10) Find the following points (n=10) from **Fig. 31** and fill in the blanks with

abbreviations (*i.e., notes within the boxes of the screen*) for body surfaces.



Q10. (1point) Based on the answers to **Q9**, which of the following statements is correct?

A. The **fpd4** region is medial to the **fpd2** region.

B. The **hpd2** region is medial to the **hpd4** region.

C. The **fl** region is rostral to the **hp** region.

D. The **fl** region is caudal to the **t** region.

E. The **mvB2** region is lateral to the **mvA3** region.

Q11. (1 point) Based on the normal S1 map, what can you conclude about the following areas?

Case	Smaller area	Larger area
A	Forelimb (**fl** + **fp** + **fpd** + **fm**)	Hindlimb (**hl** + **hp** + **hpd** + **hm**)
B	Forelimb (**fl** + **fp** + **fpd** + **fm**)	Trunk (**t**)
C	Hindlimb (**hl** + **hp** + **hpd** + **hm**)	Trunk (**t**)

D	Mystacial vibrissa (**mv**)	Rostral vibrissa (**rv**)
E	Forelimb (**fl + fp + fpd + fm**)	Vibrissa (**mv + rv**)

Q12. (1 point = 0.5 × 2) In the hindlimb region, S1 neurons receiving sensory information overlap with the motor neurons that cause muscle contraction. Find a coordinate (**unit: mm**) which supports this observation.

Press  **or** 

4.2 Change in S1 body map after digit amputation

By reducing the distance between checkpoints (**Note:** the electrode is moved along the x or y axis by the distance of 0.2 mm), a more precise map for the hindpaw region is obtained (**Fig. 32**). Surgery is performed to remove the **4th** hindpaw digit. At 4 weeks after digit amputation, a new body map is obtained (**Fig. 33**).

Q13. (4 points = 0.5 × 8) Put the cursor on the corresponding spots within **Figs. 32** and **33,** and notice where the post-amputation response is different from the normal response. For the locations where alterations occurred, fill in the appropriate table boxes with the **abbreviations** (i.e., *notes within the boxes of the screen*) for the digit numbers (you will fill in **4** boxes on each table, for a total of **8** boxes).

Normal

lateral

2.8				
2.6				
2.4				

hpd4 amputated

lateral

2.8				
2.6				
2.4				

2.2				
(mm)	−0.6	−0.8	−1.0	−1.2

→ caudal

2.2				
(mm)	−0.6	−0.8	−1.0	−1.2

→ caudal

Q14. (1 point) What changes occurred in the S1 body map after digit amputation?

Case	Activation of neurons by stimulation of	Became responsive to
A	**hpd3**	**hpd2**
B	**hpd3**	**hpd2** or **hpd5**
C	**hpd4**	**hpd2**
D	**hpd4**	**hpd3** or **hpd5**
E	**hpd5**	**hpd2** or **hpd3**

Press  **or** 

4.3 Biochemical and histological changes after digit amputation

(1) Biochemical changes (**Fig. 34**)

Glutamate is a neurotransmitter. To explore the molecular basis of S1-body-map reorganization following amputation, changes in the amount of glutamate- and GABA- receptors in S1 tissue were tracked over an extended period of time. The amount of glutamate-receptors (*green curve*) increased by 250% of control (*dotted line*) at 1 week after the 4th hindpaw digit amputation; whereas, the amount of GABA-receptors (*blue curve*) rose to 180% of control at 4 weeks post-amputation.

(2) Histological changes (**Fig. 35**)

Using transverse sections of S1 tissue, the location of glutamate- or GABA-receptors on the neuronal surface can be visualized using antibodies against those receptors. Immunostaining of the S1 cell surface (asterisks) shows that glutamate-receptors (**a** and **c**, arrows) increase at 1 week post-amputation, whereas GABA-receptors (**b** and **d**, arrows) rise at 4 weeks post-amputation.

Q15. (2 points) Based on **Figs. 33**, **34**, and **35**, choose the **incorrect** statement.

A. An increase in neuronal excitability is observed at 1 week after amputation.

B. An increase in neuronal inhibition is observed 4 weeks after amputation.

C. In the normal state, the S1 body map is maintained by a balance between excitatory sensory input and local inhibition within the cortex.

D. During 1-4 weeks after amputation, the balance between excitatory input and local inhibition is always maintained.

E. Electrophysiological changes at 4 week after amputation are accompanied by biochemical and histological changes in S1 tissue.

Hope you've got interested in Neuroscience.

Press 

Let's dissect a spider and be a Spiderman!

TASK II. (24 points) Anatomy of spider

Caution: Handle carefully, because only **one** spider will be provided for each student.

Please note that the vials are labeled Venom gland, Silk gland, Heart, and Book lung in English.

This task is composed of 2 parts.

Part I. (14 points) Exploration of the spider cephalothorax.

Q16. Both spiders and insects are members of phylum Arthropoda. In general, insects have two kinds of eyes; compound eye and single eyes (ocelli). Examine the spider specimen carefully under the microscope and answer the following questions.

Q16.1. (2 points) Record the types and total number of the spider's eyes.

Q16.2. (2 points) Generally, spider's eyes are arranged around its head in two distinct rows; i.e. the anterior and posterior rows. Within each row, the inner pair of eyes are designated as medial, while the outer pair is described as lateral (Table 1). Each eye is defined using two anatomical terms: anterior vs. posterior and medial vs. lateral. Examine the specimen and **draw** the relative position of eyes in the figure on the Answer Sheet. **Label** the drawn eyes with specific **codes** given in Table 2.

Table 1. Terms of anatomical position

Anterior	situated near or toward the head
Posterior	opposite of anterior
Medial	toward the midline of the body
Lateral	away from the midline

Table 2. Codes for spider eyes

Code	Terminology of spider eyes
AME	Anterior Medial Eye
ALE	Anterior Lateral Eye
PME	Posterior Medial Eye
PLE	Posterior Lateral Eye

Q17. Spiders can be divided into two suborders based on the positions of the cheliceral fangs. Using the forceps, examine the movement of the spider fangs under the dissecting microscope. Then, answer the following questions.

Q17.1. (1 point) What is the striking direction of the fangs?

A	from forwards to downwards
B	from downwards to forwards
C	from inside to outside
D	from side to center
E	from center to side

Q17.2. (1 point) The fang forms an articulation (or joint) with the chelicerae. What type of joint is the articulation?

A	Plane joint
B	Pivot joint
C	Hinge joint
D	Saddle joint
E	Ball-and-socket joint

Q18. (1 point) As arthropods, spiders have segmented bodies with jointed limbs. The head is composed of several segments that fuse during development. Being chelicerates, their bodies consist of two segments – the cephalothorax and the abdomen (Figure 1).

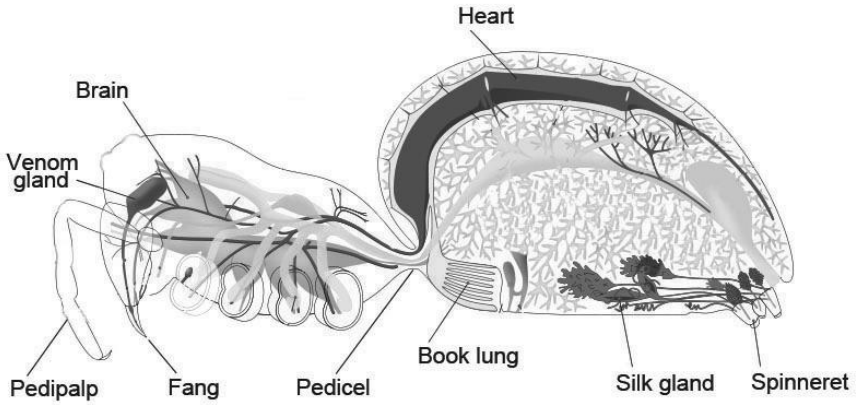


Figure 1. Diagram of spider

Which of the following (1~4) correctly represents the segmental differentiation of the cephalothorax in spiders compared to Trilobite, an ancient chelicerate?

Trilobite	1	2	3	4
Eye	Eye	Eye	Eye	Eye
A			A	A
L	C	P	C	P
L	P	C	P	C
L	L	L	L	L
L	L	L	L	L
L	L	L	L	L
L	L	L	L	L
Body	Body	Body	Body	Body

<Abbreviations>

A: Antenna, C: Chelicera, L: Leg, P: Pedipalp

Caution: From now on, you will dissect the internal organs (venom glands, silk gland, heart, and book lung) of the spider. Using the Ringer's solution provided, you need to keep the dissected organs from drying. You will be scored based on the correctness and the intactness of the preparation. Points will be deducted when there is a failure to remove the correct organ.

Q19. Most spiders possess venom that is injected into prey through the fangs of the chelicerae. Spiders have a pair of venom glands that lie either in the chelicerae or in front of the cephalothorax (see the diagram of spider in Figure 1). The venom gland consists of an outermost muscle layer, an underlying secretion layer and a duct. Locate the venom glands of the spider provided.

Q19.1. (2 points) Dissect out the pair of venom glands from the spider and put it in the vial labeled Venom gland after the following examination. It is not required to separate the venom glands from the chelicerae.

Q19.2 (1 point) Examine the outermost muscle layer of the venom gland under the microscope. What is the direction of the muscular orientation?

A	Longitudinal direction
B	Circular direction
C	Spiral direction
D	Bilateral direction
E	Irregular direction

Q20. (2 points) In most spiders, each leg has several segments and the tip of the last segment has claws. Remove the 1st and 2nd legs from the spider body. Using the microscope, count the number of segments and claws on each leg.

Q21. Many spider species exhibit sexual dimorphism. In sexually mature male spiders, the final segment of the pedipalp develops into a complicated structure that is used to transfer sperm to the female during mating. This apparatus makes the male palp so enlarged that it is often described as resembling a boxing glove.

Q21.1. (1 point) Examine the external morphology of the spider specimen provided and identify the sex of the spider.

Q21.2. (1 point) Pedipalps of spiders also have segmentation like the legs. Using the microscope, count the number of the segments and claws in each pedipalp.

Part II. (10 points) Exploration of the spider abdomen.

Q22. (1 point) The abdomen and cephalothorax of a spider are connected by a thin waist called the pedicel, which allows the abdomen to move in all directions (see the diagram of spider in Figure 1).

Which of the following organ systems does not pass through the pedicel?

A	Nervous system
B	Respiratory system
C	Circulatory system
D	Digestive system
E	Integumentary system

Q23. The silk-spinning apparatus of the spider is located at the posterior end of the ventral abdomen. This apparatus is composed of three pairs of spinnerets. Generally, the spinnerets are arranged in two distinct rows; anterior and posterior. Anatomically, the inner pair of spinnerets is defined as medial, and the outer pair is lateral (Table 3). Accordingly, the position of a spinneret is defined using these two positional terms.

Q23.1. (1 point) Compare the external morphology of the spinnerets with the following diagram. Label each spinneret in the answer sheet using the codes given in Table 3.

Table 3. Spider spinnerets

Code	Position of spinneret
A	Anterior
AM	Anterior medial
AL	Anterior lateral
P	Posterior
PM	Posterior medial
PL	Posterior lateral

Q23.2. (1 point) Identify the structure posterior to the spinneret under the microscope .

A	Anus
B	Spermatheca
C	Spiracle
D	Copulatory organ
E	Spinneret

Q24. Spiders produce various kinds of silk fibers from the silk glands. There are seven gland types in the specimen provided, each producing a different type of silk (Table 4).

Table 4. Silk glands of the spider

Code of silk gland	Number of pairs	Connection to spinneret
A	Numerous	Middle & posterior
B	2	Posterior
C	1	Posterior
D	1	Anterior
E	1	Middle
F	Numerous	Anterior
G	3	Middle (1) & posterior (2)

Q24.1. (1 point) Dragline silk is produced by the largest silk glands of this spider (Figure 2). Use the codes in Table 4 to locate the largest silk gland.

Figure 2. The silk gland which produces dragline silk

Q24.2. (2 points) Dissect one complete silk gland which produces dragline silk from this spider. After dissecting the silk gland, place the organ in the vial labeled Silk gland.

Q25. (2 points) With reference to Figure 1, dissect the heart tube from the abdomen and place it in the vial labeled Heart.

Q26. (2 points) With reference to Figure 1, locate and dissect one complete book lung. Place the organ in the vial labeled Book lung.

Country Code: _____	Student Code: _____

PRACTICAL TEST 2

Answer Sheet

PHYSIOLOGY AND ANATOMY

Total Points: 49

Duration: 90 minutes

TASK I. (25 points)

Q1. (1 point)

A	B	C	D	E

Q2. (1 point = 0.5 x 2)

p	
q	

Q3. (1 point)

	Hz

Q4. (2 points)

A	B	C	D	E

Q5. (1 point)

A	B	C	D	E

Q6. (1 point)

A	B	C	D	E

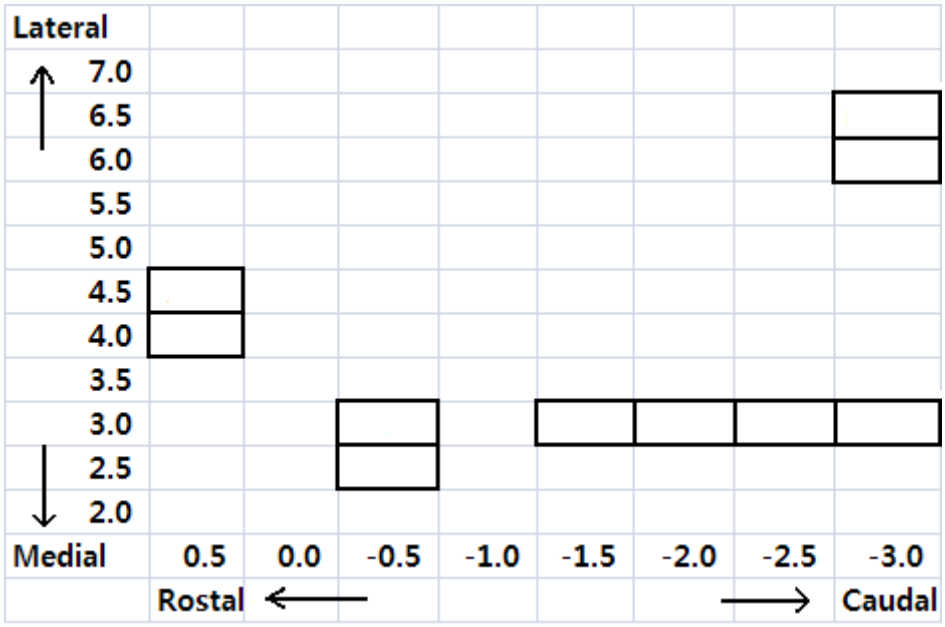
Q7. (1 point)

A	B	C	D	E

Q8. (2 points)

A	B	C	D	E

Q9. (5 points = 0.5 x 10)



Q10. (1 point)

A	B	C	D	E

Q11. (1 point)

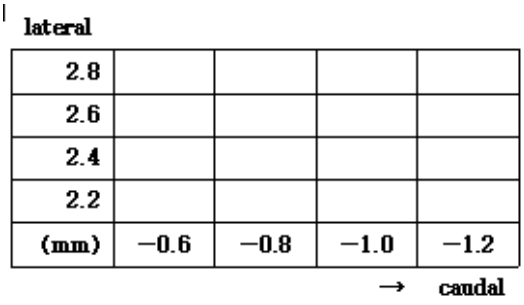
A	B	C	D	E

Q12. (1 point = 0.5 x 2)

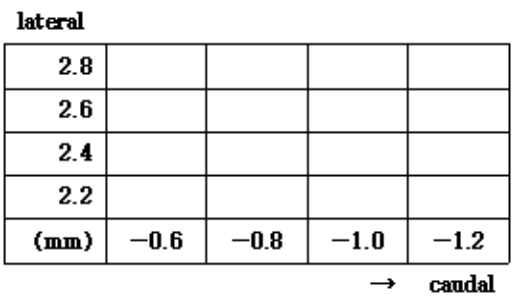
x	
y	

Q13. (4 points = 0.5 x 8)

Normal



hpd4 amputated



Q14. (1 point)

A	B	C	D	E

Q15. (2 points)

A	B	C	D	E

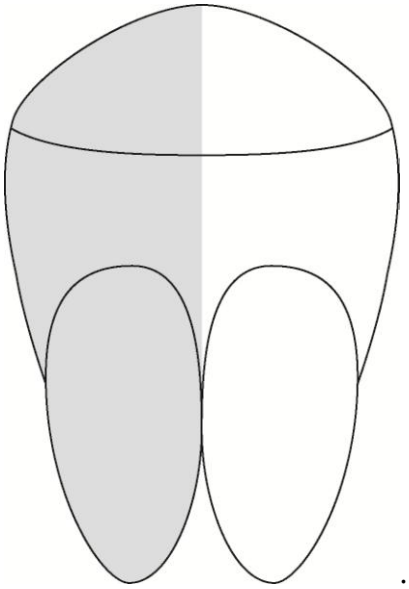
TASK II. (24 points)

Part I. (14 points)

Q16.1. (2 points = 1 × 2)

Type of eye	Total number of eye
Compound eye	
Ocellus	

Q16.2. (2 points = 0.5 × 4)



Frontal view of the head

Q17.1. (1 point)

A	B	C	D	E

Q17.2. (1 point)

A	B	C	D	E

Q18. (1 point)

1	2	3	4

Q19.1. (2 points)

(Place the organ in the provided vial, labeled **Venom gland** in English)

Q19.2. (1 point)

A	B	C	D	E

Q20. (2 points = 0.5 × 4)

	1^{st} leg	2^{nd} leg
Number of segments		
Number of claws		

Q21.1. (1 point)

Sex of the spider provided	
Male	Female

Q21.2. (1 point = 0.5 × 2)

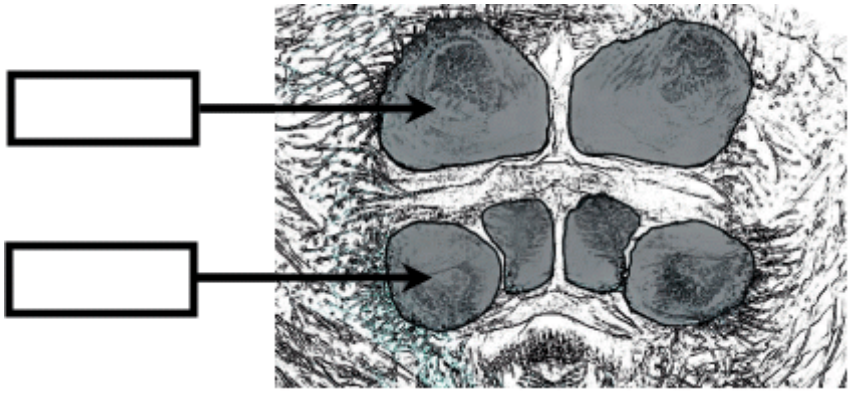
Number of the segments	
Number of claws	

Part II. (10 points)

Q22. (1 point)

A	B	C	D	E

Q23.1. (1 point = 0.5 × 2)



Q23.2. (1 point)

A	B	C	D	E

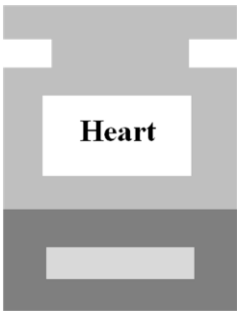
Q24.1. (1 point)

Code of silk gland	

Q24.2. (2 points)

(Place the organ in the provided vial, labeled **Silk gland** in English.)

Q25. (2 points)



(Place the organ in the provided vial, labeled **Heart** in English.)

Q26. (2 points)

(Place the organ in the provided vial, labeled **Book lung** in English.)

PRACTICAL TEST 2

Answer Key

PHYSIOLOGY AND ANATOMY

Total Points: 49

Duration: 90 minutes

TASK I. (25 points)

Q1. (1 point)

A	B	C	D	E
	√			

Q2. (1 point = 0.5 x 2)

p	8
q	20

Q3. (1 point)

40	Hz

Q4. (2 points)

A	B	C	D	E
	√			

Q5. (1 point)

A	B	C	D	E
			√	

Q6. (1 point)

A	B	C	D	E
				√

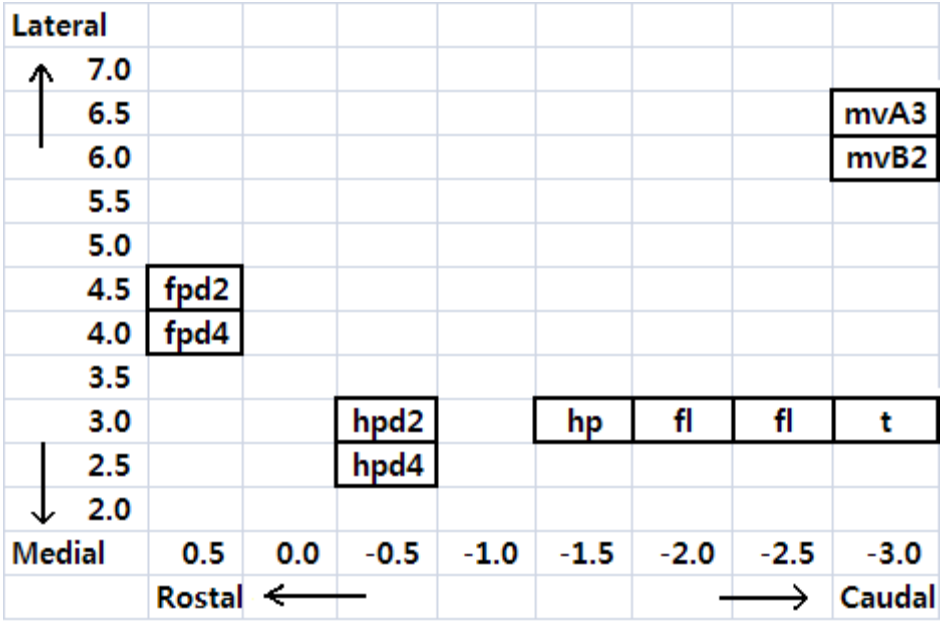
Q7. (1 point)

A	B	C	D	E
			√	

Q8. (2 points)

A	B	C	D	E
			√	

Q9. (5 points = 0.5 x 10)



1. For answers ‘mvA3’ or ‘mvB2’, ‘A3’ or ‘B2’ is evaluated as a correct answer, because the picture of the rat shows A3 or B2 only.

Q10. (1 point)

A	B	C	D	E
√				

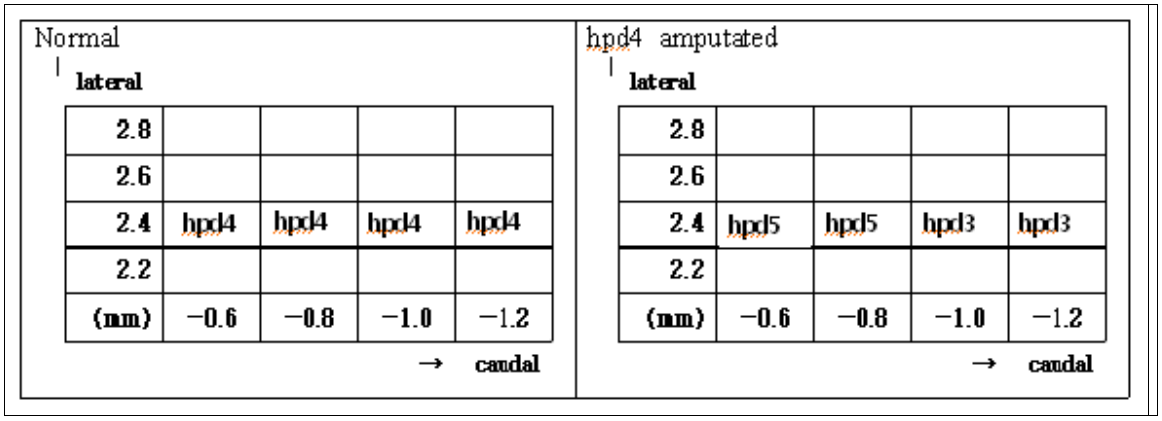
Q11. (1 point)

A	B	C	D	E
				√

Q12. (1 point = 0.5 x 2)

X	0
Y	2.5

Q13. (4 points = 0.5 x 8)



1. 'Numbers' without 'hpd' are also evaluated as correct answers, because the picture of the rat contains digit numbers only.

Q14. (1 point)

A	B	C	D	E
			√	

Q15. (2 points)

A	B	C	D	E
			√	

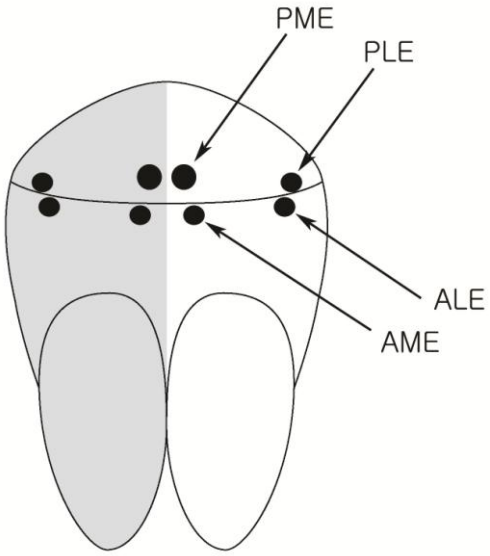
TASK II. (24 points)

Part I. (14 points)

Q16.1. (2 points = 1 × 2)

Type of eye	Total number of eye
Compound eye	0
Ocellus	8

Q16.2. (2 points)



Frontal view of the head.

1. 1 point will be given if you draw 4 pairs of eyes at proper position.
   1 point to the 4 correct codes (1 point = 0.25 × 4).
2. 1 point will be given if you draw 4 pairs of eyes at proper position without correct codes or with incorrect codes.
3. 0 point will be given if you draw incorrect number of eyes.

Q17.1. (1 point)

A	B	C	D	E
			√	

Q17.2. (1 point)

A	B	C	D	E
		√		

Q18. (1 point)

①	②	③	④
√			

Q19.1. (2 points)

(Place the organ in the provided vial, labeled **Venom gland** in English)

1. 2 points will be given if you dissect out a pair of venom glands with or without chelicerae.
2. 1 point will be given if you dissect out the chelicerae with one venom gland.
3. 0 point will be given if you dissect incorrect organ.

Q19.2. (1 point)

A	B	C	D	E
		√		

Q20. (2 points = 0.5 × 4)

	1st leg	2nd leg
Number of segments	7	7
Number of claws	3	3

Q21.1. (1 point)

Sex of the spider provided	
Male	Female
	√

Q21.2. (1 point = 0.5 × 2)

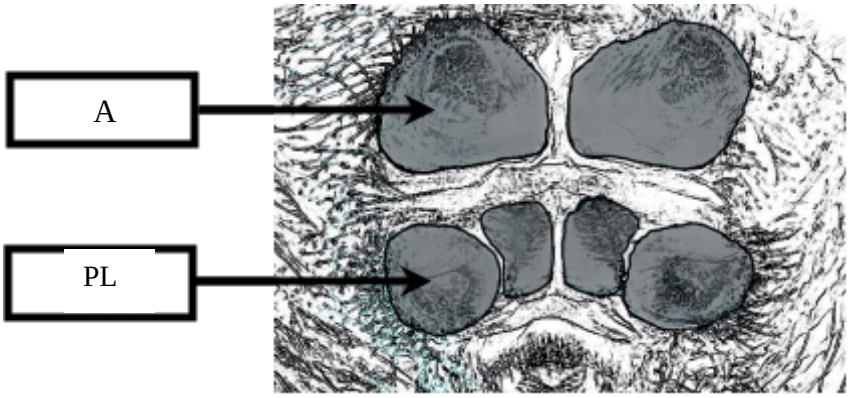
Number of the segments	6
Number of claws	1

Part II. (10 points)

Q22. (1 point)

A	B	C	D	E
	√			

Q23.1. (1 point = 0.5 × 2)



Q23.2. (1 point)

A	B	C	D	E
√				

Q24.1. (1 point)

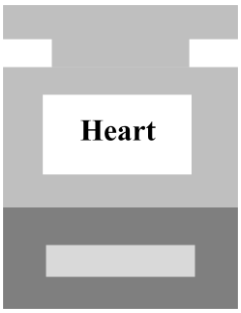
Code of silk gland	D

Q24.2. (2 points)

(Place the organ in the provided vial, labeled **Silk gland** in English.)

1. 2 points will be given if you dissect proper silk gland with both regions of ampulla and tail.
2. 1 point will be given if you dissect proper silk gland with ampulla region only.
3. 0 point will be given if you dissect incorrect silk gland.

Q25. (2 points)



(Place the organ in the provided vial, labeled **Heart** in English.)

Q26. (2 points)

(Place the organ in the provided vial, labeled **Book lung** in English.)

Country Code: _____	Student Code: _____

The 21st INTERNATIONAL BIOLOGY OLYMPIAD

11th – 18th July, 2010

Changwon, KOREA

PRACTICAL TEST 3

GENETICS AND CELL BIOLOGY

Total Points: 50

Duration: 90 minutes

Dear Participants,

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

  Task I (35 points)

  (1) Study of promoter-driven regulation of gene expression. (20 points)

  (2) Characterization of the relationship between genotypes and phenotypes (15 points)**.**

  Task II: Observation of meiotic cells in preserved rye anthers (15 points)

- 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 hand.

- Stop answering and put down your pencil **immediately** after the end bell rings. The supervisor will collect the Question Paper and the Answer Sheet.

Good Luck!!

GENETICS AND CELL BIOLOGY

This practical test is composed of 2 tasks.

TASK I. (35 points)

(1) Study of the promoter-driven regulation of gene expression

(2) Characterization of the relationship between genotypes and phenotypes

This task is composed of 2 parts.

Materials and Equipments

On individual Table

1. Fluoro-spectrophotometer
2. Microfuge tubes containing 50 μL each of nine differently-labeled plant extracts; two identically labeled tubes are provided for each type of extract (2 × 9 = 18 tubes). The transparent tubes are for the protein assay, and the black tubes are for fluorescence measurements.

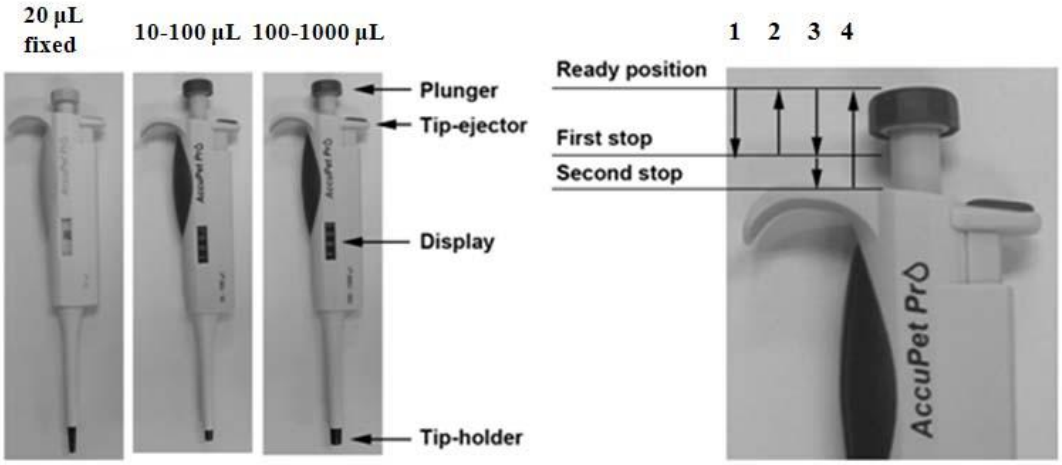
Label	Treatment	Label	Treatment
WT-0	Plant WT + distilled water		
WT-1	Plant WT + 1 μM hormone H	WT-100	Plant WT + 100 μM hormone H
dA-1	Plant dA + 1 μM hormone H	dA-100	Plant dA + 100 μM hormone H
dAB-1	Plant dAB + 1 μM hormone H	dAB-100	Plant dAB + 100 μM hormone H
dABC-1	Plant dABC + 1 μM hormone H	dABC-100	Plant dABC + 100 μM hormone H

3. 12 mL Bradford reagent in a 15 mL plastic tube (Bradford reagent is used to determine concentration of protein)
4. 1 mL of 1 mM MUG (fluorescence substrate to measure GUS activity) in a microfuge tube
5. 12 mL of stop reagent for the GUS (enzyme β-glucuronidase which converts MUG into MU) reaction in a 15 mL plastic tube
6. Two DNA size-marker tubes (labeled M, 50 μL each) and eight tubes containing *Eco*RI-digested DNA (labeled P1~P8, 50 μL each)
7. Two microfuge tubes labeled as GUS BL and Pro BL, respectively.
8. Three micropipettes (one each for 10-100 μL and 100-1000 μL, and a fixed volume pipette for 20 μL)
9. A box of yellow tips for the 20 μL and the 10-100 μL micropipettes
10. A box of blue tips for the 100-1000 μL micropipette
11. A DNA electrophoresis apparatus, equipped with a 1% agarose gel in 1X TAE gel running buffer. If your gel is broken, raise your hand for assistance.
12. A tip disposal container
13. Polygloves
14. 25 cuvettes for the Fluoro-spectrophotometer
15. A calculator
16. A timer
17. A Scotch tape
18. An ice bucket filled with ice
19. Microfuge tube racks
20. Green card

<u>On the common equipment table</u>

1. Gel documentation system equipped with a UV source

Handling of Micropipettes



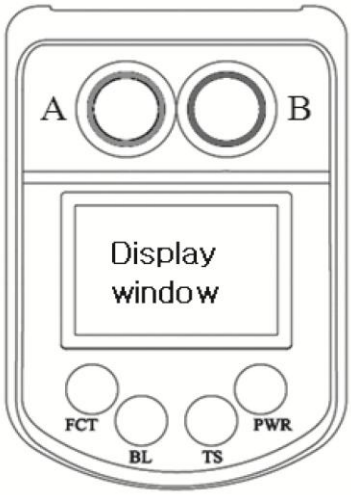
Adjustment method

Turn the plunger to set the volume to the desired value, which can be seen in the display window. Remember that each micropipette has designated range of volumes as indicated on the pipette. Do not exceed the limits of this range.

Usage method

1) Secure the pipette tip to the tip holder. Gently push down the plunger to the first stop.
2) Hold and lower the tip down into the solution to a depth of 2~4 mm. Release the plunger slowly to allow it to return to its original position.
3) Remove the pipette from the liquid, and transfer the contents to the desired tube. Push the plunger to the first stop and then push further to the second stop to discharge the solution completely from the tip.
4) Remove the pipette from the tube and release the plunger. Eject the used tip into the tip disposal container by pressing the tip-ejector.

Operating Instruction for the Fluoro-Spectrophotometer (measures fluorescence of MU and absorbance of proteins at 595 nm)



A: Cuvette holder for protein measurement
B: Cuvette holder for fluorescence of MU measurement
FCT: Function key
BL: Blank key
TS: Test sample key
PWR: Power key

Usage method

Important: Please be sure not to touch the light path of cuvettes.

1) Press the PWR (⏻) button to turn on the machine. The display window will be turned on after a beep.
2) To set the blank sample to zero, insert the blank cuvette in an appropriate holder (use cuvette holder A to measure protein concentration, and cuvette holder B to measure GUS activity). The cuvette indicator will be turned on (for the holder A and for the holder B).

Note: Two blank samples for measurement of GUS activity and amounts of proteins are provided in the microfuge tubes labeled as GUS BL and Pro BL, respectively.

3) Press the BL button, and the blank indicator (⍗) will appear when the blank is set at 0.0.
4) To measure a sample, remove the blank cuvette and insert the test cuvette in the same cuvette holder, and press the TS button. The result will be displayed after 5-10 seconds, and the indicator will appear in the display window ( )
5) To end the machine, keep the PWR button pressed till beep is heard.

Operating Instruction for the DNA Gel Electrophoretic Apparatus

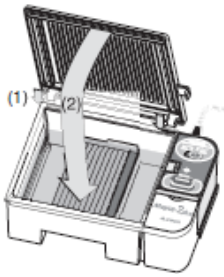
1) Load the samples to the wells using the 20 μL micropipette.

2) After verifying that the operation switch of the power supply is OFF, close the migration tank lid.

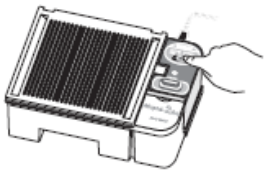
Do this as follows:

(1) First, insert the 2 tabs on the cover into the holes in the migration tank.

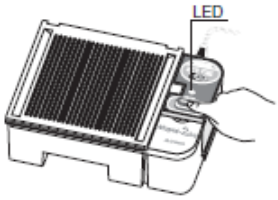
(2) Then, rotate the cover forward to close it.



3) Set the voltage to “Half” using the output selection switch.



4) Push the operation switch to start the migration.

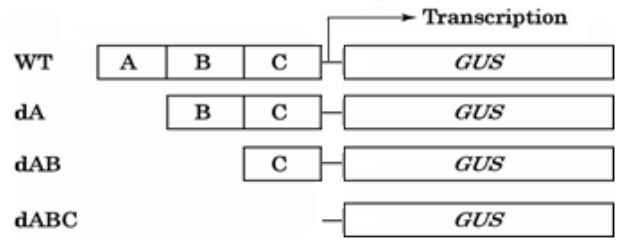


5) In this experiment, the gel running time should be 30 min. Make sure to turn the operation switch OFF when the running is finished.

Part I. (20 points) Using the gene *X*-fused GUS reporter gene to analyze hormonal effects on gene expression and to characterize the hormone-responsive elements in the promoter.

Plants respond to their hormones by regulating hormone-responsive genes. Within a gene promoter, a specific DNA sequence(s), the *cis*-element, dictates the proper time and amount of gene expression. Regulation is primarily controlled by an hormone-responsive transcription factor(s) that binds specifically to this region, resulting either in gene activation or suppression.

In this task, you will examine the mode of hormonal regulation in the hormone-responsive gene *X* of *Arabidopsis*. To find the hormone-responsive regions, in the promoter and to understand the mode of hormonal regulation of gene *X* expression, the promoter of gene *X* is divided into A~C (each of these domain may function as enhancer, silencer or minimal promoter). Then, a variety of *Arabidopsis* transgenic plants expressing the GUS (β-glucuronidase) reporter gene under the control of the different regions of the promoter, as diagramed below, was generated. The GUS will be produced when the promoter of gene *X* is activated. The GUS enzyme converts MUG into MU, and its activity can be measured by quantifying MU fluorescence using a fluoro-spectrophotometer.



< Four *Arabidopsis* transgenic plants carrying different reporter constructs>

Q1. The purpose of the first experiment is two-fold: (1) to find the promoter region containing a hormone-responsive *cis*-element and (2) to investigate the effects of different hormone H concentrations on gene *X* expression. All transgenic plants (WT, dA, dAB, and dABC) were treated with either 1 μM or 100 μM of hormone H. To assess the level of GUS expression, plant extracts were prepared from these treated plants. (See the table in the materials and method section.)

Using the methods described in the next section, measure the fluorescence value and absorbance at 595 nm of each 50 μL plant extracts. Based on these measurements, calculate the amount of MU (nmole MU/50 μL plant extracts), the amount of proteins (μg/50 μL plant extracts), and the resulting GUS activity (nmole MU/μg protein/min) for each extract. Record your results in Table 1 in the answer sheet to find answers for **Q1.1**, **Q1.2**, and **Q1.3.**

Measurement of fluorescence and determination of MU amount

1)-1. Turn on and set the fluoro-spectrophotometer to zero with 500 μL of the blank sample labeled GUS BL.

1)-2. Take a microfuge tube of plant extracts (each tube contains 50 μL extracts) prepared from each WT-O or hormone-treated transgenic plant, and **mix well** (by gentle tapping) with 50 μL of 1 mM MUG solution. Start with tube labeled WT-O and proceed in an order shown in the table in Materials and Equipments.

1)-3. Incubate the reaction mixtures at room temperature for 10 min.

1)-4. Stop the reaction by adding 900 μL of stop reagent (1M sodium carbonate in GUS extraction buffer) into each 100 μL reaction solution in the same order you added MUG. **Mix well** by tapping.

1)-5. Take 500 μL of the finished mixture from each tube, and measure the fluorescence using the fluoro-spectrophotometer.

1)-6. Calculate the amount of MU in the sample using the formula provided below. Record the fluorescence value and the calculated amount of MU in Table 1 in the answer sheet. This is the amount of MU produced from each of 50 μL plant extracts.

$Y = 0.04 X + 2.5$

Y: the amount of MU (nmoles/ 50 μL plant extracts)

X: the measured fluorescence value [from step 1)-5]

Measurement of absorbance at 595 nm and determination of protein amount

2)-1. Turn on and set the fluoro-spectrophotometer to zero with 500 μL of the blank sample labeled Pro BL.

2)-2. Take a microfuge tube with extracts (each tube contains 50 μl extracts) prepared from each WT-O or hormone-treated transgenic plant, and mix well with 950 μL of Bradford reagent. Incubate at room temperature for 5 min.

2)-3. Take 500 μL of the reaction mixture from each tube, and measure the absorbance at 595 nm using the fluoro-spectrophotometer.

2)-4. Calculate the amount of proteins using the formula provided below. Record the absorbance at 595 nm and the calculated amount of proteins in Table 1 in the answer sheet. This is the amount of proteins contained in each of 50 μL plant extracts.

$Y = 98X + 2.8$

Y: the amount of protein (μg/50 μL plant extracts) X: the measured absorbance at 595 nm of the solution [from step 2)-3]

Calculation of GUS activity

3)-1. Considering that this GUS enzyme reaction was performed for 10 min [refer to 1)-3], calculate GUS activity in nmole MU/μg protein/min and record the value in Table 1 in the answer sheet.

Table 1 is worth of 9 points.

Q1.1. (4 points) Based on your results in <Table 1>, put a checkmark (√) in the appropriate box of each plant in Table **Q1.1** in the answer sheet.

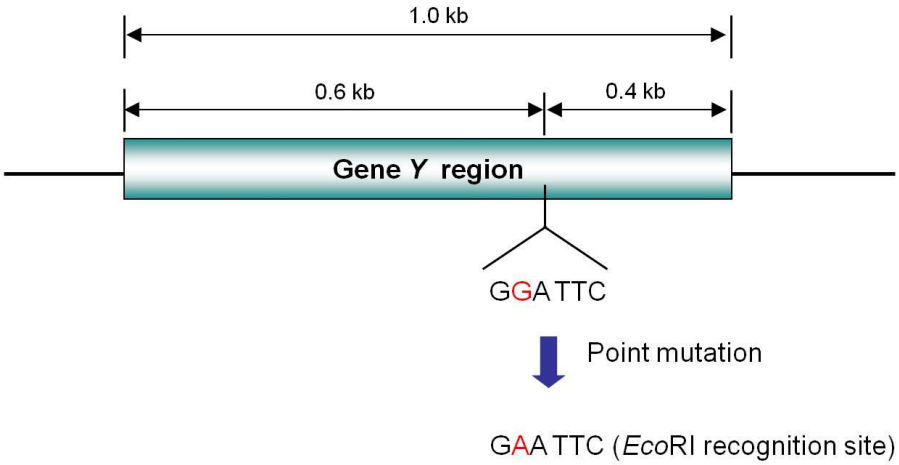
Note: - stimulation: more than **3-fold** increase in gene *X* expression
- no effect: less than **3-fold** increase in gene *X* expression

Q1.2. (6 points = 2 × 3) Based on your previous conclusions in **Q1.1**, determine the regulatory function (enhancer, silencer, or minimal promoter) of each *cis*-element (A~C). Put a checkmark (√) in the appropriate box in Table **Q1.2** in the answer sheet.

Q1.3. (1 point) How does 100 μM of hormone H regulate the expression of gene *X*? Based on your finding from <Table 1>, determine the mode of action of hormone H. Put a checkmark (√) in the appropriate box in Table **Q1.3** in the answer sheet.

Part II. (15 points) A co-relationship analysis between genotype and phenotype, and the prediction of gene pool frequencies using Hardy-Weinberg mathematics.

Q2. Gene *Y* encodes a protein that regulates plant growth. The schematic figure below depicts the region of gene *Y* in genomic DNA and a point mutation within.



There are eight plants with homozygous (*YY* or *yy*) or heterozygous (*Yy*) genotype, showing either wild type or dwarf phenotypes (*Y*: wild type allele, *y*: mutant allele. The alleles *Y* and *y* do not specify whether they are dominant or reccessive). To analyze the genotype of these plants, the

1 kb region of gene *Y* was amplified by PCR. This fragment was then digested with *Eco*RI restriction enzyme, which cuts GAATTC sequence. Other than the *Eco*RI site created by the point mutation, there is no other *Eco*RI recognition sequence in gene *Y*. Using the protocol described below, perform a gel electrophoresis of the *Eco*RI-digested PCR products.

Genotyping of gene *Y* by gel electrophoresis

Note: Always wear polygloves during the experiment !!!

(1) A total of ten microfuge tubes are provided: two DNA size marker tubes (M) and eight tubes containing *Eco*RI-treated PCR product from Plants 1~8 (P1~P8, respectively). Starting from left, in the order of M, P1~P8, M, load 20 μL out of 50 μL DNA solution into each well of a prepared agarose gel in the electrophoresis apparatus. Use the 20 μL micropipette to load samples. Change pipette tip for each sample.

Note: The DNA size marker solution contains 0.4, 0.6, and 1.0 kb DNA fragments. DNA loading buffer and DNA-staining dye are already included in each tube.

(2) Refer to <Operating instructions for DNA gel electrophoretic apparatus> to put the cover on the electrophoresis apparatus, to turn on the apparatus, and to run the electrophoresis.

Note: Upon starting the electrophoresis, make sure that the output indicator LED is lit and that bubbles are forming on the platinum electrodes.

(3) Run the gel for 30 min at "Half" voltage.

* **IMPORTANT: While the gel is running, proceed to TASK II !!!**

(4) Turn off the apparatus. Then, raise the **green card** to request help for photography of the agarose gel.

Note: The assistant will bring a gel transfer box to you. Make sure that your student code is on the box.

(5) When you receive the agarose gel picture, attach it to Q2.1 of the answer sheet using Scotch tape. Label the number of each plant (P1~P8) on each lane of the gel picture.

(6) In Table Q2.2 in the answer sheet, put checkmarks (√) to designate the size of DNA fragments and the genotype of each plant.

Q2.1. (3 points) Attach the agarose gel picture to a space given on the answer sheet. And label the number of each plant (P1~P8) on each lane of the gel picture.

Q2.2. (4 points) Determine the size of DNA fragment(s) and the genotype (*YY*, *Yy* or *yy*) of each plant. Put a checkmark (√) in the appropriate box in Table **Q2.2** in the answer sheet.

Q2.3. (2 points) Based on the genotype and phenotype of each plant given in **Q2.2**, deduce the characteristic of the mutation. Put a checkmark (√) in the appropriate box in the Table **Q2.3** in the answer sheet.

Q2.4. (2 points) If you cross Plant 1 with Plant 3 (from **Q2.2**), what is the probability (%) that an offspring will be a dwarf plant? Write your answer in the answer sheet.

Q2.5. (4 points) The eight plants in Q2.2 represent a population. If this population produces 10,000 plants in the next generation, what would be the expected number of heterozygous and dwarf offspring, respectively? (Assume that this population is in Hardy-Weinberg equilibrium.)

TASK II. (15 points) Observation of meiotic cells in preserved rye anthers

Materials, instruments and tools	Numbers
1. Light microscope with objective lenses of 4X, 10X, 40X, and 100X	1
2. Preserved rye anthers in a vial	2
3. Dissecting needle set	1
4. Slides and cover slips	5 each
5. Filter paper (7 cm diameter)	3
6. Forceps	1
7. Ceramic tile	1
8. Petri-dish (6 cm diameter)	1
9. Acetocarmine solution with a dropper	1
10. Pencil	1
11. Eraser	1
12. Disposable plastic pipet	1
13. Red card	1

Background

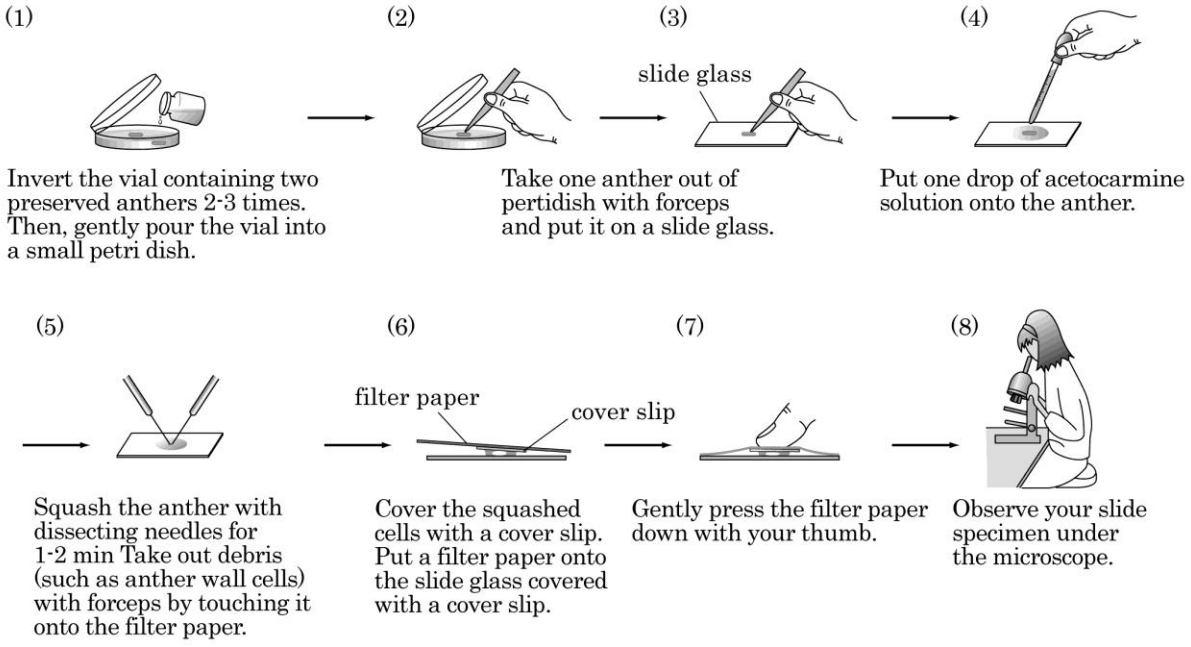
Using a light microscope, you will observe meiotic cells in preserved rye anthers. Anthers at a specific stage of meiosis were selected and were preserved in 70% ethanol.

Requirements – Overview

Using the microscope, identify anther cells undergoing meiosis. In the space given in the answer sheet, sketch an image of meiotic cell you observe at 400X magnification (**Q3.2**)

Procedure

1) Before you start observation, check for the presence of two small preserved anthers in the vial.
2) Take out the ceramic tile out of the tray, and put one glass slide on it.
3) Observe your specimen under the microscope at 100X magnification, and find at least one cell undergoing meiosis. Then, observe **one** cell at 400X magnification and draw this image in the given area of the answer sheet (Q3.2). Make sure that this cell is at the center of your field of view. After you finish drawing, raise the **red card**. The lab assistant will come to you and will take a photograph of the slide.



< Procedure for observation of meiotic cells in preserved rye anthers>

Notes :

1. In step (1), if the anthers won't come out, put the solution back into the vial using the disposable plastic pipet and repeat step (1).
2. Be careful not to break the anther in step (2).
3. You may use a filter paper to remove excess 70% ethanol in step (3).
4. Do not press too hard, or you may break the cells and/or the cover slip in step (7).
5. You are provided with two anthers to prepare your specimen. If you fail to make good specimen with the first anther, please repeat the procedure and make another preparation using the other. However keep in mind that the time for your experiment is limited.

Q3. Answer the following questions.

Important: You will see two types of cells under the microscope as shown in Figure Q3. The circled ones are examples of cells undergoing meiosis, and the rest are cells of the anther wall.

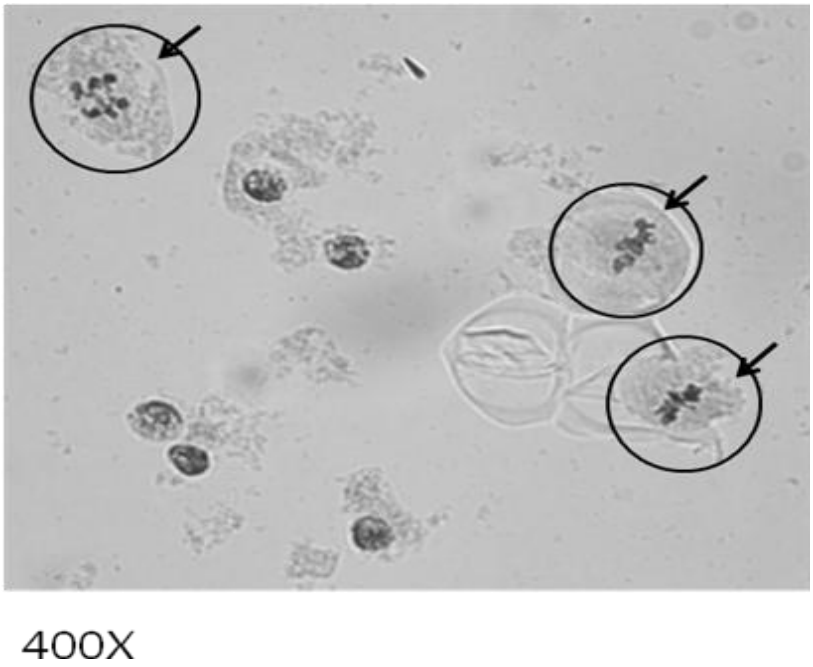


Figure Q3. Examples of cells undergoing meiotic cell division observed under a microscope.

Q3.1. (1 point) What kind of cells in the anther undergoes meiosis? Put a checkmark (√) in the appropriate box in the answer sheet.

Q3.2. (8 points) Draw **<u>one cell</u>** undergoing meiosis at 400X magnification in the answer sheet. Do not label the drawing.

Important : This cell must be at the center of your field of view when the picture is taken.

Q3.3. (4 points) At what meiotic stage are the cells? Put a checkmark (√) in the appropriate box in the answer sheet.

Q3.4. (2 points) What is the amount of DNA in the cell undergoing meiosis that you observed and a cell of the anther wall, respectively? Put checkmarks (√) in the appropriate boxes in the answer sheet.

Country Code: _____	Student Code: _____

PRACTICAL TEST 3

Answer Sheet

GENETICS AND CELL BIOLOGY

Total Points: 50

Duration: 90 minutes

TASK I. (35 points)

Part I. (20 points)

Table 1. (9 points=1X9)

<Table 1> GUS expression, after treatment with different concentrations of hormone H, in transgenic plants containing various deletions within the gene *X* promoter

Plants	Measured fluorescence [value from 1)-5]	Amount of MU* produced by 50 μL plant extracts [nmole MU, value from 1)-6]	Measured absorobance at 595 nm [value from 2)-3]	Amount of proteins* in 50 μL plant extracts [μg, value from 2)-4]	GUS activity* [nmole MU/μg protein/min, value from 3)-1]
WT-0 (control)					
WT-1					
dA-1					
dAB-1					
dABC-1					
WT-100					
dA-100					
dAB-100					
dABC-100					

* The calculated values should be rounded to the nearest hundredth.

Q1.1. (4 points = 0.5 x 8)

Plant treated with hormone H	Effect of hormone treatment in plants	
	Stimulation	No effect
WT-0	Control	
WT-1		
dA-1		
dAB-1		
dABC-1		
WT-100		
dA-100		
dAB-100		
dABC-100		

Q1.2. (6 points = 2 x 3)

Region in gene *X* promoter	Function (enhancer, silencer, or minimal promoter)		
	enhancer	silencer	minimal promoter
A			
B			
C			

Q1.3. (1 point)

Action mode	
Transcriptional positive feedback regulation	
Transcriptional negative feedback regulation	

Part II. (15 points)

Q2.1. (3 points)

<Attach the agarose gel picture here>

Q2.2. (4 points = 0.5 x 8)

Plant	Size of the DNA fragment(s) (kb)			Genotype			Phenotype
	0.4	0.6	1.0	*YY*	*Yy*	*yy*	
Plant 1							Wild type
Plant 2							Wild type
Plant 3							dwarf
Plant 4							dwarf
Plant 5							Wild type
Plant 6							Wild type
Plant 7							Wild type
Plant 8							dwarf

Q2.3. (2 points)

Characteristic of the mutation	Dominant	
	Recessive	

Q2.4. (2 points)

Probability of dwarf offspring	(%)

Q2.5. (4 points = 2 x 2)

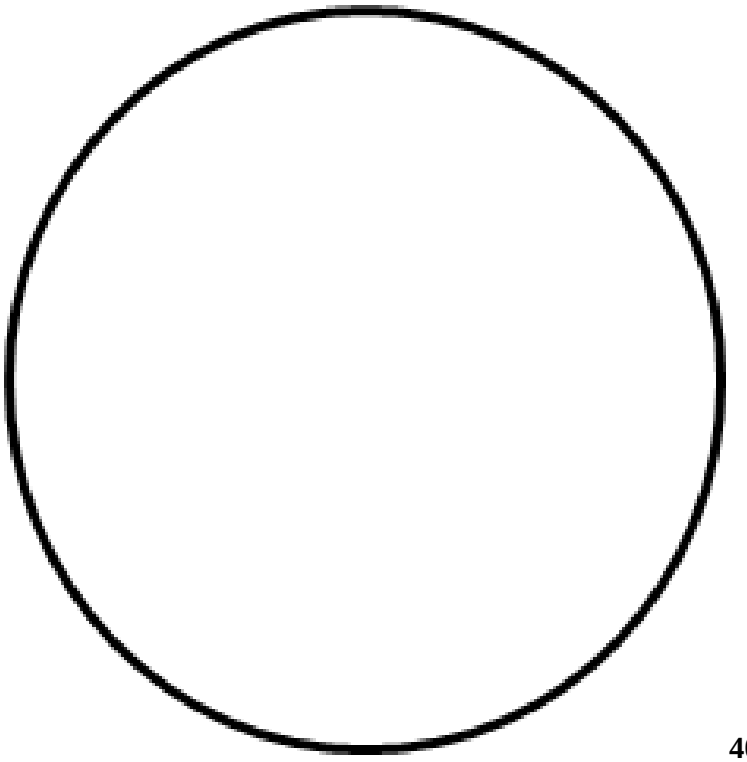
Number of heterozygous (*Yy*) offspring	
Number of dwarf offspring	

TASK II. (15 points)

Q3.1. (1 point)

synergid cells	
egg cells	
megaspore mother cells	
Pollen (microspore)mother cells	
pollen	
antipodal cells	

Q3.2. (8 points)



400X

Q3.3. (4 points)

Meiosis I				Meiosis II			
Prophase	Metaphase	Anaphase	Telophase	Prophase	Metaphase	Anaphase	Telophase

Q3.4. (2 points = 1 × 2)

	The amount of DNA	
	The cell undergoing meiosis	Cells constituting anther wall
1C		
2C		
3C		
4C		

C: the amount of DNA in a haploid complement

PRACTICAL TEST 3

Answer Key

GENETICS AND CELL BIOLOGY

Total Points: 50

Duration: 90 minutes

]TASK I. (35 points)

Part I. (20 points)

Table 1. (9 points = 0.2 × 45)

<Table 1> GUS expression, after treatment with different concentrations of hormone H, in transgenic plants containing various deletions within the gene X promoter

Plants	Measured fluorescence [value from 1)-5]	Amount of MU* produced by 50 μL plant extracts [nmole MU, value from 1)-6]	Measured absorbance at 595 nm [value from 2)-3]	Amount of proteins* in 50 μL plant extracts [μg, value from 2)-4]	GUS activity* [nmole MU/μg protein/min, value from 3)-1]
WT-0 (control)	600-900	26.5-38.5	0.5-0.7	51.8-71.4	0.04-0.07
WT-1	6,000-9,000	242.5-362.5	0.5-0.7	51.8-71.4	0.34-0.70
dA-1	6,000-9,000	242.5-362.5	0.5-0.7	51.8-71.4	0.34-0.70
dAB-1	600-900	26.5-38.5	0.5-0.7	51.8-71.4	0.04-0.07
dABC-1	600-900	26.5-38.5	0.5-0.7	51.8-71.4	0.04-0.07
WT-100	600-900	26.5-38.5	0.5-0.7	51.8-71.4	0.04-0.07
dA-100	6,000-9,000	242.5-362.5	0.5-0.7	51.8-71.4	0.34-0.70
dAB-100	600-900	26.5-38.5	0.5-0.7	51.8-71.4	0.04-0.07
dABC-100	600-900	26.5-38.5	0.5-0.7	51.8-71.4	0.04-0.07

1. A correct answer for each measurement or calculation within ranges indicated in the Table 1 is worth of 0.2 point.

Q1.1. (4 points = 0.5 x 8)

Plant treated with hormone H	Effect of hormone treatment in plants	
	Stimulation	No effect
WT-0	Control	
WT-1	√	
dA-1	√	
dAB-1		√
dABC-1		√
WT-100		√
dA-100	√	
dAB-100		√
dABC-100		√

1. Answers that are not supported by the GUS activity data in Table 1 will be considered as wrong answers.
2. Plural choices for each hormone-treated plant are null.

Q1.2. (6 points = 2 x 3)

Region in gene *X* promoter	Function (enhancer, silencer, or minimal promoter)		
	enhancer	silencer	minimal promoter
A		√	
–B	√		
C			√

1. An answer that is not supported by the data in Table 1 and **Q1.1** will be considered as a wrong answer.
2. Plural choices for each promoter region are null

Q1.3. (1 point)

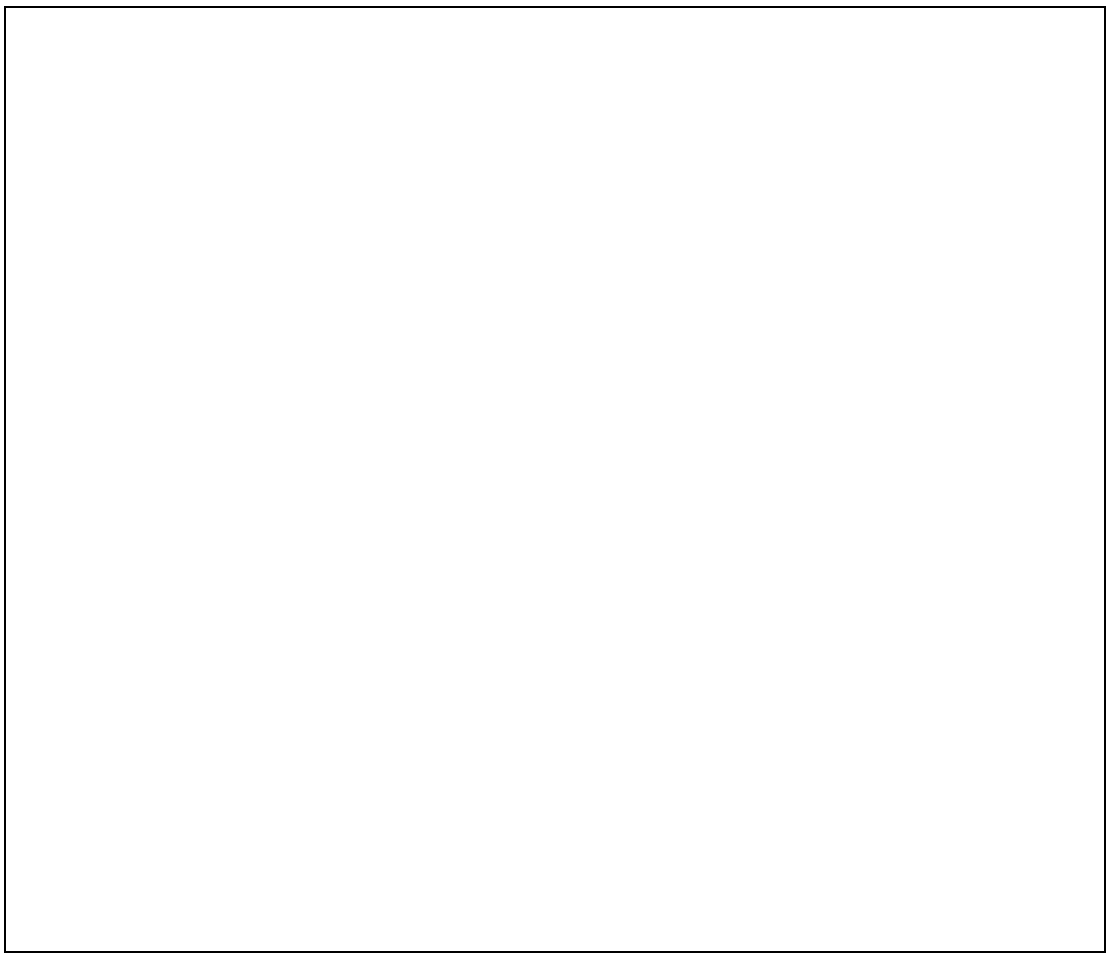
Action mode	
Transcriptional positive feedback regulation	
Transcriptional negative feedback regulation	√

1. The answer that is not supported by the answers for WT-0, WT-1 and WT-100 in Table 1 and **Q1.1** will be considered as a wrong answer.

Part II. (15 points)

Q2.1. (3 points)

<Attach the agarose gel picture here>



1. **<u>3 points</u>** :
   At the least one of the two DNA marker lanes was loaded, together with <u>ALL</u> of the plant samples.
2. **<u>2 points</u>** :
   1) Both marker lanes were loaded but one of the plant sample is missing.
      Or
   2) All plant samples were loaded but both of the marker lanes are missing.

   In all cases, electrophoresis should be performed long enough to allow the genotyping. Otherwise no point will be given.

Q2.2. (4 points = 0.5 x 8)

Plant	Size of the DNA fragment(s) (kb)			Genotype			Phenotype
	0.4	0.6	1.0	*YY*	*Yy*	*yy*	
Plant 1			√	√			Wild type
Plant 2			√	√			Wild type
Plant 3	√	√	√		√		dwarf
Plant 4	√	√	√		√		dwarf
Plant 5			√	√			Wild type
Plant 6			√	√			Wild type
Plant 7			√	√			Wild type
Plant 8	√	√				√	dwarf

Q2.3. (2 points)

Characteristic of the mutation	Dominant	√
	Recessive	

Q2.4. (2 points)

Probability of dwarf offspring	50 (%)

Q2.5. (4 points = 2 x 2)

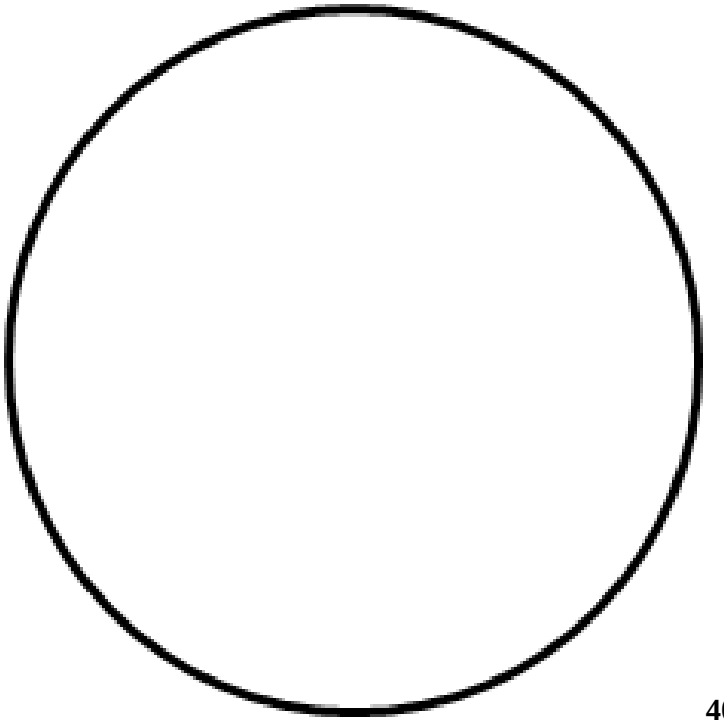
Number of heterozygous (*Yy*) offspring	3750
Number of dwarf offspring	4375

TASK II. (15 points)

Q3.1. (1 point)

synergid cells	
egg cells	
megaspore mother cells	
pollen (microspore) mother cells	√
pollen	
antipodal cells	

Q3.2. (8 points)



400X

******

1. 8 points will be given if a cell undergoing meiosis with proper chromosome is drawn and photo evidence is available.
2. 4 points will be given if drawing is good but photo evidence is not available.
3. 2 points will be given if only anther wall cell(s) were drawn.
4. 0 point will be given if i) no cell is drawn, ii) no chromosomes is discernable, iii) only cell debris are drawn.

Q3.3. (4 points)

Meiosis I				Meiosis II			
Prophase	Metaphase	Anaphase	Telophase	Prophase	Metaphase	Anaphase	Telophase
√							

1. 4 point will be given if the meiotic stage drawn in Q3.2 is correctly checked.

Q3.4. (2 points = 1 × 2)

	The amount of DNA	
	The cell undergoing meiosis	Cells constituting anther wall
1C		
2C		√
3C		
4C	√	

C: the amount of DNA in a haploid complement

Country Code: _____	Student Code: _____

The 21st INTERNATIONAL BIOLOGY OLYMPIAD

11th – 18th July, 2010

Changwon, KOREA

PRACTICAL TEST 4

ECOLOGY

Total Points: 51

Duration: 90 minutes

Dear Participants,

- In this test, you have been given the following 4 tasks:

  Task I: Characteristics of Coastal Animal Communities (16 points)

  Task II: Mark and Recapture Method (8 points)

  Task III: Interspecific Interaction (14 points)

  Task IV: Prey Choice Model (13 points)

- 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 hand.

- Stop answering and put down your pencil **immediately** after the end bell rings. The supervisor will collect the Question Paper and the Answer Sheet.

Good Luck!!

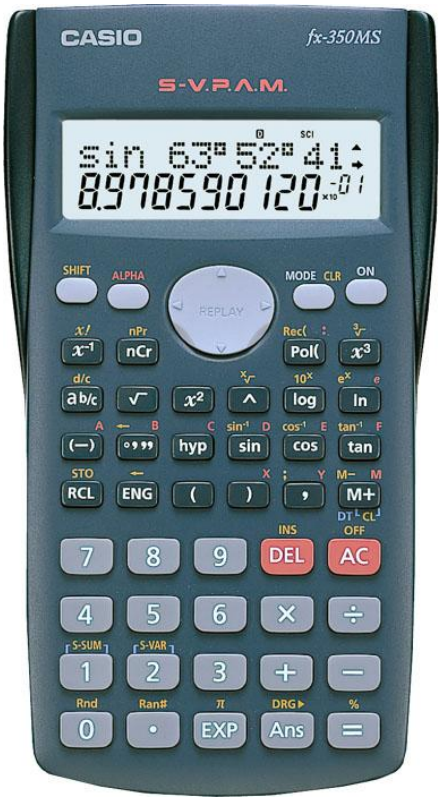
TASK I. (16 points) Characteristics of coastal animal communities

Materials	Quantity
1. Community model board (40 x 37 cm)	1
2. Transparent quadrat board (37 x 37 cm)	1
3. Electronic calculator	1

Introduction

A population is defined as a group of individuals of a single species inhabiting a specific area, and a community is a group of populations of different species inhabiting a specific area. Identification of the characteristics of populations and communities is a basic element in predicting ecological change due to environmental factors.

Using Calculator



1. Press **ON** to turn on the calculator
2. Calculation Examples

   To calculate 1 + 1 , press **1 + 1 =**

   To calculate ln 90 (= $\log_e$ 90), press **ln 9 0 =**

   To calculate $\sqrt{\frac{2^2}{5^2}}$, press $\sqrt{\ }$ **(2** x^2 **) ab/c (5** x^2 **) =**

3. To correct characters, move the cursor by pressing ◄ or ►, and press **DEL** to delete the character or **SHIFT DEL** to insert character
4. To clear all of the calculation you have entered, press **AC**.
5. Press **Shift AC** to turn off the calculator. Calculator will automatically turn off if you do not perform any operation for about 10 minutes.

Q1. (4 points) The model provided on the board is a coastal community consisting of nine animal species. Determine the population size (abundance, N) of each species in the community using a complete enumeration survey and the population density (per unit area, 1 m^2) of each species. The size of each quadrat is 1 m x 1 m. Round values to the nearest hundredth (two decimal places) during your calculations, and record the values in the answer sheet.

Species	Population size
Starfish 	
Razor clam 	
Sea slater 	**15**
Sea urchin 	
Fiddler crab 	**13**
Octopus 	
Oyster 	
Mudskipper 	
Sea anemone 	**13**

Q2. (2 points) The table below records species' population sizes in two different coastal communities. Calculate 'the proportion of relative abundance' of each species. Round values to the nearest hundredth (two decimal places) during your calculations, and record the values in the answer sheet.

Community A		Community B	
Species	Population size	Species	Population size
Starfish	13	Fiddler crab	2
Razor clam	18	Barnacle	18
Sea slater	13	Sea anemone	15
Sea urchin	12	Sea cucumber	2
Fiddler crab	11	Hermit crab	5
Gastropod	8	Gastropod	8
Oyster	12		
Mudskipper	9		
Sea anemone	10		
Total	106	Total	50

Q3. (4 points) A rank-abundance curve is a chart that displays the species in a community ordered from most abundant to rare based on relative abundance. Using the relative abundances you previously calculated (in Q2), make a rank-abundance curve for each community on the grid-line in the answer sheet. Indicate community A as 'A' and community B as 'B' on the curve, and write appropriate titles and scales for the X-axis and the Y-axis.

Q4. (4 points) Calculate the Shannon-Wiener species diversity index (H′) for each of the two coastal communities using the following equation. Round values to the nearest hundredth (two decimal places) during your calculations. Put the values in the box in the answer sheet.

$$H' = -\sum_{i=1}^{n}(p_i \ln p_i)$$

where,

p_i = the proportion of the i^{th} species

ln p_i = the natural logarithm of p_i

n = the number of species in the community

Q5. (1 point) Which statement is/are correct for your rank-abundance curves? Put checkmark(s) (√) in all appropriate boxes in the answer sheet.

A. Species evenness is higher in community A than in community B.

B. Species evenness is lower in community A than in community B.

C. Species richness is higher in community A than in community B.

D. Species richness is lower in community A than in community B.

Q6. (1 point) Which statement is correct for the species diversity index of the two communities? Put a checkmark (√) in the appropriate box in the answer sheet.

A. The area with the higher diversity index (H') should be conserved.

B. The species diversity index (H') indicates the species number inhabiting the coastal area.

C. The species diversity index (H') is inversely proportional to species evenness in an area.

D. The species diversity index (H') depends on both species richness and species evenness.

TASK II. (8 points) Mark and recapture method

Materials	Quantity
1. Pottery with beads	1
2. Sampling net (100 ml)	1
3. Electronic calculator	1

Introduction

A few individuals are captured, marked and released back into the population. The population is sampled again and the numbers of marked individuals in this sample counted. Assuming an equal recapture rate for all individuals and without repetitive counting of the same individual, the population size can be simply estimated by using a modified Lincoln Index as follows:

$$N = \frac{(M+1)(S+1)}{(R+1)} - 1$$

N: Estimation of population size

M: Number of individuals marked

S: Number of individuals captured in the second sample

R: Number of marked individuals recaptured

In this task, the pottery represents a pond with a diving beetle population (the beads). One bead represents one diving beetle. This population contains 40 individuals marked with a red sticker that had been captured during the first sampling. You will be performing the second sampling of this population.

Q7. (4 points) Using the sampling net, capture a sample of diving beetles from the pond (the second sampling). Take two full scoops and combine them. (Assume this population does not have birth, death, emigration, or immigration of individuals between the first and the second sampling events). Estimate population size to the nearest tenth (one decimal place) and record your result in the answer sheet.

Q8. (4 points) The mark and recapture method has a degree of uncertainty because it is an estimation by sampling, not by a total population count. We can measure uncertainty through the calculation of standard error (SE). Standard error (SE) can be obtained by the function given below.

$$SE = \sqrt{\frac{M^2(S+1)(S-R)}{(R+1)^2(R+2)}}$$

The 95% confidence interval can be obtained by this calculation: N ± t · SE. The 95% confidence interval means that the size of original population is within the range of the confidence interval with 95% certainty. The *t*-value is the Student's *t*- value when the degree of freedom is infinity. (At infinity, the Student's *t*-value is also refer to as Z-value). The critical values of the Student's *t* distribution are provided.

Find the appropriate *t* in the table and calculate SE and the 95 % confidence interval for your estimate of population size. Enter the numbers you obtain in the table in the answer sheet. Round your value to the nearest hundredth (two decimal places) during your calculations and record your values in the answer sheet.

Critical Values of the Student's *t* Distribution

Degree of freedom	$\alpha = p = P(t > t_{critical})$			
	0.1	0.05	0.01	0.001
1	6.31	12.71	63.66	636.62
2	2.92	4.30	9.93	31.60
3	2.35	3.18	5.84	12.92
4	2.13	2.78	4.60	8.61
5	2.02	2.57	4.03	6.87
6	1.94	2.45	3.71	5.96
7	1.89	2.37	3.50	5.41
8	1.86	2.31	3.36	5.04
9	1.83	2.26	3.25	4.78
10	1.81	2.23	3.17	4.59
11	1.80	2.20	3.11	4.44
12	1.78	2.18	3.06	4.32
13	1.77	2.16	3.01	4.22
14	1.76	2.14	2.98	4.14
15	1.75	2.13	2.95	4.07
16	1.75	2.12	2.92	4.02
17	1.74	2.11	2.90	3.97
18	1.73	2.10	2.88	3.92
19	1.73	2.09	2.86	3.88
20	1.72	2.09	2.85	3.85
21	1.72	2.08	2.83	3.82
22	1.72	2.07	2.82	3.79
23	1.71	2.07	2.82	3.77
24	1.71	2.06	2.80	3.75
25	1.71	2.06	2.79	3.73
26	1.71	2.06	2.78	3.71
27	1.70	2.05	2.77	3.69
28	1.70	2.05	2.76	3.67
29	1.70	2.05	2.76	3.66
30	1.70	2.04	2.75	3.65
40	1.68	2.02	2.70	3.55
60	1.67	2.00	2.66	3.46
120	1.66	1.98	2.62	3.37
∞	1.65	1.96	2.58	3.29

TASK III. (14 points) Interspecific interaction

Materials	Quantity
1. Two species model board (30 × 32 cm)	1
2. Transparent quadrat board (30 × 30 cm)	1
3. Electronic calculator	1

Introduction

Spiral shellfishes and clams live in the same habitat. In order to know whether there is an interaction between these two species, we examine the distribution of each species in that habitat.

Q9. (2 points) Using the given quadrat board, observe whether the spiral shellfish and the clam are absent and/or present in each quadrat. Write the number of quadrats you have observed in the box in the answer sheet.

Q10. (2 points) The significance of the species' distributions measured in this habitat can be examined by using the Chi-square (χ^2) test. The null hypothesis for the χ^2 test in this situation is that the distribution of each species:

A. is nonrandom.

B. is independent of each other.

C. shows a mutually negative influence.

D. shows a mutually positive influence.

E. is influenced by a third species.

Put a checkmark (√) in the appropriate box in the answer sheet.

Q11. (4 points) To perform the χ^2 test, first determine the expected counts for each observational class. For example, the expected counts of quadrats where both species are present is calculated by multiplying the number of quadrats where one species is present with the number of quadrats where the other species is present divided by the total number of quadrats. Compute the other expected counts similarly to the nearest tenths (one decimal place) and fill the table in the answer sheet.

Q12. (2 points) Using the function below, calculate the χ^2 value for this data set. Record your value to the nearest hundredth (two decimal places) in the answer sheet.

$$\chi 2 = \sum \frac{(\text{observed count} - \text{expected count})^2}{\text{expected count}}$$

Q13. (1 point) In order to evaluate the Chi-square value (χ^2), the degree of freedom for the data set must be determined (*df*). What is the degree of freedom for this data set? Record the value in the answer sheet.

Q14. (2 points) Decide whether to reject or not reject the null hypothesis using the significance level (probability, p) of 0.05. In the given χ^2 table, locate the degree of freedom in the appropriate column. Compare your calculated χ^2 test statistic to the tabular χ^2 value to make your decision. Put a checkmark (√) in the appropriate box in the answer sheet.

Q15. (1 point) Considering the spatial pattern of the distribution, what kind of interactionis likely to be taking place between the two species? Choose **all** possible options and put a checkmark (√) in the appropriate box in the answer sheet.

A. No interaction

B. Commensalism

C. Competition

D. Parasitism

E. Exclusion

Chi-square Table

Degree of freedom	Probability, p				
	0.99	0.95	0.05	0.01	0.001
1	0.000	0.004	3.84	6.64	10.83
2	0.020	0.103	5.99	9.21	13.82
3	0.115	0.352	7.82	11.35	16.27
4	0.297	0.711	9.49	13.28	18.47
5	0.554	1.145	11.07	15.09	20.52
6	0.872	1.635	12.59	16.81	22.46
7	1.239	2.167	14.07	18.48	24.32
8	1.646	2.733	15.51	20.09	26.13
9	2.088	3.325	16.92	21.67	27.88
10	2.558	3.940	18.31	23.21	29.59
11	3.05	4.58	19.68	24.73	31.26
12	3.57	5.23	21.03	26.22	32.91
13	4.11	5.89	22.36	27.69	34.53
14	4.66	6.57	23.69	29.14	36.12
15	5.23	7.26	25.00	30.58	37.70
16	5.81	7.96	26.30	32.00	39.25
17	6.41	8.67	27.59	33.41	40.79
18	7.02	9.39	28.87	34.81	42.31
19	7.63	10.12	30.14	36.19	43.82
20	8.26	10.85	31.41	37.57	45.32
21	8.90	11.59	32.67	38.93	46.80
22	9.54	12.34	33.92	40.29	48.27
23	10.20	13.09	35.17	41.64	49.73
24	10.86	13.85	36.42	42.98	51.18
25	11.52	14.61	37.65	44.31	52.62
26	12.20	15.38	38.89	45.64	54.05
27	12.88	16.15	40.11	46.96	55.48
28	13.57	16.93	41.34	48.28	56.89
29	14.26	17.71	42.56	49.59	58.30
30	14.95	18.49	43.77	50.89	59.70

TASK IV. (13 points) Prey choice model

	Quantity
Materials	
1. Prey model board (22 × 24 cm)	2
2. Electronic calculator	1

Introduction

A foraging animal encounters various types of prey items. Each type of prey can be characterized by its energy content (E), the time required to search for that prey (searching time, Ts), and the time required to capture and consume it (handling time, Th). Therefore, we can measure prey profitability by the function E/(Ts+Th). In this situation, according to optimality theory, natural selection would favor behaviors that maximize an animal's net energy intake per amount of foraging time.

The behavioral options for a forager are whether to accept or to reject an item of a given prey type when it is encountered. Assume that there are two kinds of prey item, Type 1 and Type 2. Let the profitability be higher for Type 1 — that is, $E_1/(Ts_1+Th_1) > E_2/(Ts_2+Th_2)$. Thus, Type 1 items should always be accepted. Prey profitability is density-dependant. That is, profitability of a prey species changes if the prey species becomes less abundant.

On the boards for Site I and Site II, there are three prey items for gulls:

Prey A: Spiral shellfish 

Prey B: Clam 

Prey C: Razor clam 

Q16. (2 points) For Site I, record the density of each of the prey species A, B, C (number of individuals per m^2, assuming that each quadrat is 1m x 1m). Calculate searching time (Ts) for each of the prey species, where the species-specific searching time at density = 1 has been provided. Ts= (1/density)·*a* (sec). The value '*a*' is a species-specific constant. Calculate the values to nearest hundredth (two decimal places).

Prey species	Ts (sec) when the prey density is 1
Prey A	**10**
Prey B	**15**
Prey C	**5**

Q17. (2 points) After capturing a prey item, gulls fly high and drop the item to break its shell. The forager repeats the behavior if the shell does not break. The table below indicates the drop height and the average number of drops required at that height to break the prey's shell. For each prey type, indicate with a checkmark (√), in the answer sheet, the optimal drop height that gulls should choose, if they are optimal foragers.

	Height of drop (m)	Average number of drops required to break shell
Prey A	2	60
	3	40
	5	20
	10	8
	15	7

	Height of drop (m)	Average number of drops required to break shell
Prey B	2	60
	3	20
	5	7
	10	5
	15	4

	Height of drop (m)	Average number of drops required to break shell
Prey C	2	30
	3	10
	5	8
	10	5
	15	4

Q18. (2 points) Gulls fly one meter up or down in 0.5 seconds. Given the optimal drop height for each prey species, calculate the handling time (Th) for each prey item. Record the number in the box in the answer sheet.

Q19. (3 points) The table below lists the average energy gain from eating an individual of each prey species (kilojoules (KJ) per prey). Calculate the profitability of each prey species at Site I to the nearest hundredth (two decimal places), and record the number in the box in the answer sheet.

Prey species	Energy (KJ per prey)
Prey A	7
Prey B	25
Prey C	5

Q20. (2 points) Of the following choices, what would be the optimal decision for the gulls at site I? Put a checkmark (√) in the most appropriate box in the answer sheet.

A. Eat all of prey A.

B. Eat all of prey B.

C. Eat all of prey C.

D. Eat prey A at first and then switch to prey B.

E. Eat prey B at first and then switch to prey C.

Q21. (2 points) A gull finds an item of prey C in Site II. The gull can, however, decide not to take this item and fly to Site I where it can search for prey B. Given that Site I requires 50 seconds of flying time from Site II, what should the gull do in order to maximize the profitability of the next prey item, if it is an optimal forager? Distribution of the prey items in Site II has been provided to you. Put a checkmark (√) in the most appropriate box in the answer sheet.

A. The gull will eat the prey C in Site II.

B. The gull will move to Site I to search for prey B.

C. The gull will search for prey B in Site II.

D. The gull will move to site I to search for prey C.

E. The gull will search for prey A in Site II.

Country Code: _____	Student Code: _____

PRACTICAL TEST 4

Answer Sheet

ECOLOGY

Total Points: 51

Duration: 90 minutes

TASK I. (16 points)

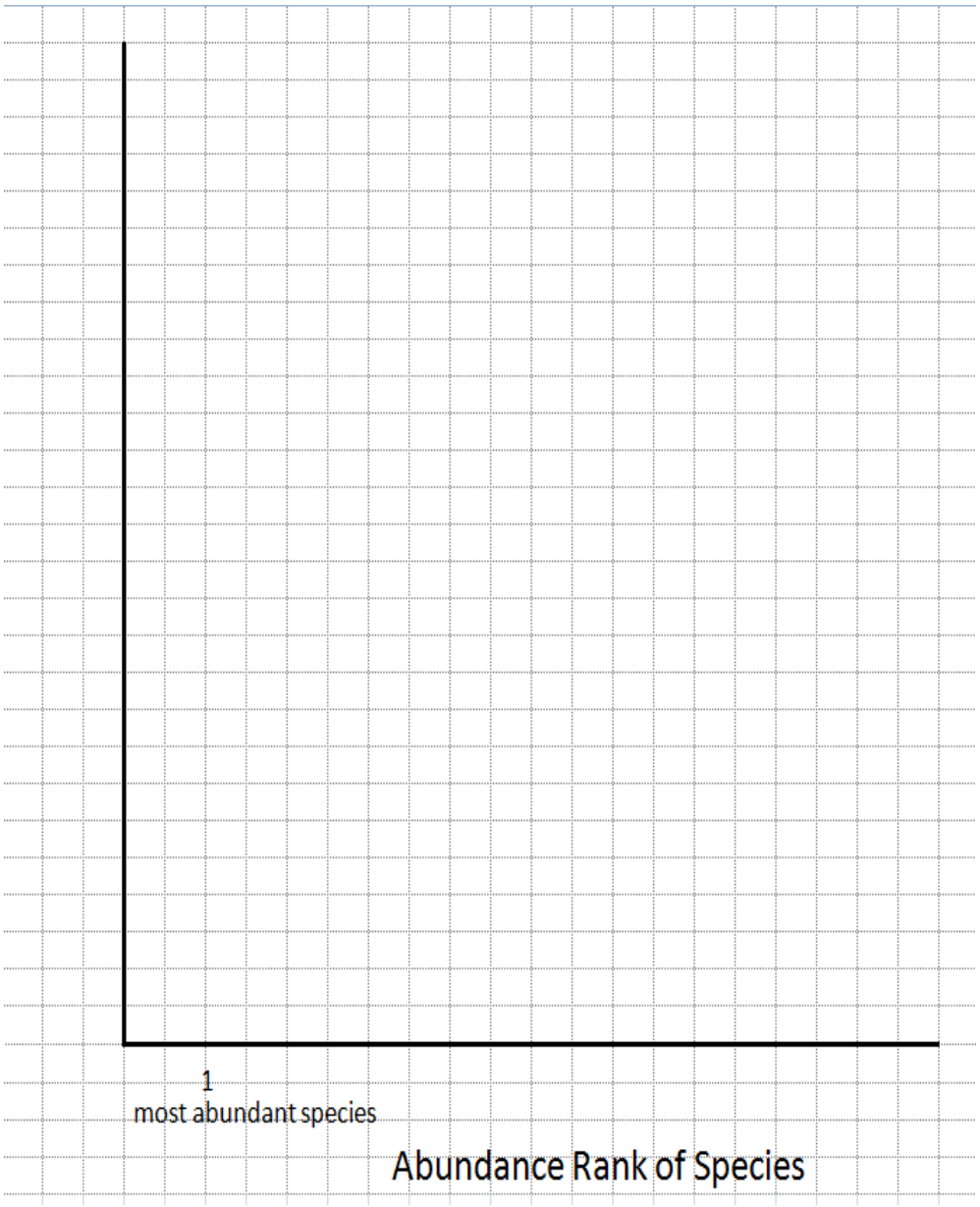
Q1. (4 points)

Species	Population size	Density (number of individuals/m^2)
Starfish 		
Razor clam 		
Sea slater 	**15**	
Sea urchin 		
Fiddler crab 	**13**	
Octopus 		
Oyster 		
Mudskipper 		
Sea anemone 	**13**	

Q2. (2 points)

Community A			Community B		
Species	Population size	Proportion of relative abundance	Species	Population size	Proportion of relative abundance
Starfish	**13**		Fiddler crab	**2**	
Razor clam	**18**		Barnacle	**18**	
Sea slater	**13**		Sea anemone	**15**	
Sea urchin	**12**		Sea cucumber	**2**	
Fiddler crab	**11**		Hermit crab	**5**	
Gastropod	**8**		Gastropod	**8**	
Oyster	**12**				
Mudskipper	**9**				
Sea anemone	**10**				
Total	**106**		Total	**50**	

Q3. (4 points)



Q4. (4 points)

Species diversity index of community A (H'_A)	Species diversity index of community B (H'_B)

Q5. (1 point)

A	B	C	D

Q6. (1 point)

A	B	C	D

TASK II. (8 points)

Q7. (4 points)

Number of individuals captured during the second sampling	
Number of marked individuals recaptured	
Estimate of the population size	

Q8. (4 points)

t-value	
SE	
Confidence interval of the estimated population size	

TASK III. (14 points)

Q9. (2 points)

Observed count		Spiral shellfish 	
		Present	Absent
Clam 	Present		
	Absent		

Q10. (2 points)

A	B	C	D	E

Q11. (4 points)

Expected count		Spiral shellfish	
		Present	Absent
Clam	Present		
	Absent		

Q12. (2 points)

χ^2	

Q13. (1 point)

Degree of freedom (*df*)	

Q14. (2 points)

	Fail to reject	Reject
Null hypothesis		

Q15. (1 point)

A	B	C	D	E

TASK IV. (13 points)

Q16. (2 points)

Prey species	Density (number of individuals/m^2)	Ts (sec) when the prey density is 1	Ts (sec) at the Site I
Prey A		**10**	
Prey B		**15**	
Prey C		**5**	

Q17. (2 points)

	Height of drop (m)	Average number of drops required to break shell	Optimal height for handling
Prey A	2	60	
	3	40	
	5	20	
	10	8	
	15	7	

	Height of drop (m)	Average number of drops required to break shell	Optimal height for handling
Prey B	2	60	
	3	20	
	5	7	
	10	5	
	15	4	

	Height of drop (m)	Average number of drops required to break shell	Optimal height for handling
Prey C	2	30	
	3	10	
	5	8	
	10	5	
	15	4	

Q18. (2 points)

Prey species	Handling time per prey (sec)
Prey A	
Prey B	
Prey C	

Q19. (3 points)

Prey species	Energy (KJ per prey)	Prey profitability
Prey A	**7**	
Prey B	**25**	
Prey C	**5**	

Q20. (2 points)

A	B	C	D	E

Q21. (2 points)

A	B	C	D	E

PRACTICAL TEST 4
Answer Key

ECOLOGY

Total Points: 51

Duration: 90 minutes

TASK I. (16 points)

Q1. (4 points)

Species	Population size	Density (number of individuals/m^2)
Starfish 	15	0.31
Razor clam 	20	0.41
Sea slater 	**15**	0.31
Sea urchin 	13	0.27
Fiddler crab 	**13**	0.27
Octopus 	10	0.20
Oyster 	14	0.29
Mudskipper 	11	0.22
Sea anemone 	**13**	0.27

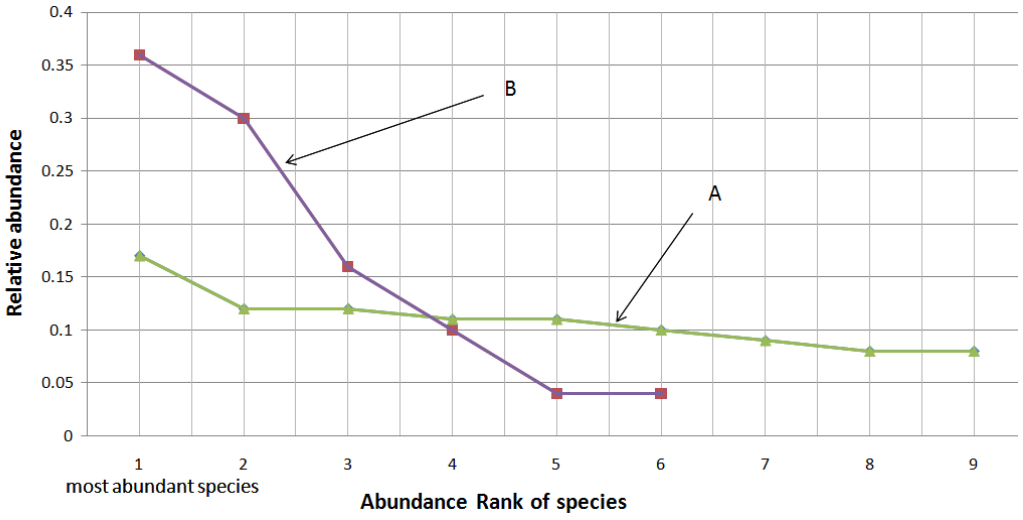
1. One point will be subtracted for any error in rounding value and error in decimal place.
2. In case of calculation error for any value, one point is subtracted for each error.
3. Only one point is subtracted for incorrect answers within each row of the table.

Q2. (2 points)

Community A			Community B		
Species	Population size	Proportion of relative abundance	Species	Population size	Proportion of relative abundance
Starfish	**13**	0.12	Fiddler crab	**2**	0.04
Razor clam	**18**	0.17	Barnacle	**18**	0.36
Sea slater	**13**	0.12	Sea anemone	**15**	0.30
Sea urchin	**12**	0.11	Sea cucumber	**2**	0.04
Fiddler crab	**11**	0.10	Hermit crab	**5**	0.10
Gastropod	**8**	0.08	Gastropod	**8**	0.16
Oyster	**12**	0.11			
Mudskipper	**9**	0.09			
Sea anemone	**10**	0.09			
Total	**106**		Total	**50**	

1. 0.5 point is subtracted for any error in rounding value and error in decimal place.
2. In case of calculation error for any value, 0.5 point is subtracted for each error.

Q3. (4 points)



1. Full points will be given for marks on the appropriate curve, and appropriate titles and scales for the Y-axis.
2. Full points are given if participant make appropriate graph using data in Q2.
3. For incorrect marks or no marks, 2 points are subtracted.
4. For no title or scales on Y-axis, 2 points are subtracted.

Q4. (4 points)

Species diversity index of community A (H'_A)	Species diversity index of community B (H'_B)
2.15	1.51

1. Full points will be given for values between 2.10 – 2.19 for community A and 1.50-1.59 for community B.
2. 2 points for each value.
3. 1 point are substracted for each error in rounding value and decimal place.
4. Full Points are given if participant make appropriate calculation using data in Q2.

Q5. (1 point)

A	B	C	D
√		√	

1. 1 point is given if participant make appropriate answer using the graph of Q3.
2. No point is given if participant mark only one out of both answers.

Q6. (1 point)

A	B	C	D
			√

TASK II. (8 points)

Q7. (4 points)

Number of individuals captured during the second sampling	Participant's Value
Number of marked individuals recaptured	Participant's Value
Estimate of the population size	Use of the Excel Table

1. 1 point will be given if the participant wrote the first and second answers.
2. 0.5 point is subtracted if the participant did not round off the numbers.
3. 0.5 point is subtracted if the participant did not record one decimal place or recorded more decimal places.

Q8. (4 points)

t-value	1.96
SE	Use of the Excel Table
Confidence interval of the estimated population size	Use of the Excel Table

1. Values found in Q.1 must be applied.
2. 0.5 point is subtracted if the participant did not round off the numbers.
3. 0.5 point is subtracted if the participant did not record one decimal place or recorded more decimal places.
4. It is OK if the confidence interval is written in the range (X~X') or in the form of Y±Y' or Y'.
5. 1 point is subtracted when the participant wrote one wrong answer.
6. Confidence interval is accepted for the point within the range of the excel calculation ±0.05

TASK III. (14 points)

Q9. (2 points)

Observed count		Spiral shellfish 	
		Present	Absent
Clam 	Present	15	12
	Absent	6	16

1. 1 point is subtracted if the participant wrote one wrong answer.

Q10. (2 points)

A	B	C	D	E
	√			

1. Plural choice is null.

Q11. (4 points)

Expected count		Spiral shellfish	
		Present	Absent
Clam	Present	11.6	15.4
	Absent	9.4	12.6

1. 1 point is subtracted for each wrong answer.
2. 0.5 point is subtracted if the participant did not round off the numbers.
3. 0.5 point is subtracted if the participant did not record one decimal place or recorded more decimal places.
4. Use the excel table for the evaluation when the observation counts are wrong (full point, in the case of exact calculation).

Q12. (2 points)

χ^2	3.96 or 3.89

1. Use the excel table for the evaluation when the answers of Q9 and Q11 are wrong (full point in the case of exact calculation).
2. Full point is given if the value is within the range of ± 0.05

Q13. (1 point)

Degree of freedom (*df*)	1

Q14. (2 points)

	Fail to reject	Reject
Null hypothesis		√

If Q12 < 3.84, "fail to reject" is a correct answer.

Q15. (1 point)

A	B	C	D	E
	√		√	

1. 1 point is obtained if the participant chose only B or D.
2. If answer of Q14 is "fail to reject", correct answer is "A".

TASK IV. (13 points)

Q16. (2 points)

Prey species	Density (number of individuals/m^2)	Ts (sec) when the prey density is 1	Ts (sec) at the Site I
Prey A	1.00	**10**	10
Prey B	0.75	**15**	20
Prey C	0.50	**5**	10

1. 0.5 point is subtracted if the participant did not round off the numbers.
2. 0.5 point is subtracted if the participant did not record one decimal place or recorded more decimal places.
3. 1 point is subtracted if the participant wrote one or two wrong answer within each row in the table.

Q17. (2 points)

	Height of drop (m)	Average number of drops required to break shell	Optimal height for handling
Prey A	2	60	
	3	40	
	5	20	
	10	8	√
	15	7	

	Height of drop (m)	Average number of drops required to break shell	Optimal height for handling
Prey B	2	60	
	3	20	
	5	7	√
	10	5	
	15	4	

	Height of drop (m)	Average number of drops required to break shell	Optimal height for handling
Prey C	2	30	
	3	10	√
	5	8	
	10	5	
	15	4	

Q18. (2 points)

Prey species	Handling time per prey (sec)
Prey A	80
Prey B	35
Prey C	30

1. 1 point is subtracted if the participant wrote one wrong answer.

Q19. (3 points)

Prey species	Energy (KJ per prey)	Prey profitability
Prey A	**7**	0.08
Prey B	**25**	0.45
Prey C	**5**	0.13

1. 1 point is subtracted if the participant wrote one wrong answer.
2. 0.5 point is subtracted if the participant did not round off the numbers.
3. 0.5 point is subtracted if the participant did not record one decimal place or recorded more decimal places.
4. Use the excel table for the evaluation using answers of Q16 and Q18 (full point, in the case of exact calculation).

Q20. (2 points)

A	B	C	D	E
				√

Q21. (2 points)

A	B	C	D	E
		√		

Plural choice is null.