Country _____	Competitor# __ __ __

16th International Biology Olympiad

Beijing

July 2005

Practical Examination

Part I

Total time available: 90 minutes

The 16th IBO Practical Tests

First name:

Last name

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Code:

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Practical tests Part I:

Biochemistry and Molecular Biology

Very important notice: you should start task 1 first and while the gel electrophoresis is running, start and finish task 2.

Task 1: Separation of plasmid DNA restriction fragments by Agarose Gel Electrophoresis (24 points

Instruments: Centrifuge, Agarose gel electrophoresis apparatus and Fluorescence gel imaging systems.

Important:

Raise the blue card on the bench table to ask for help when you want to use the electrophoresis power supplies.

Introduction

Plasmids are circular double-stranded DNA molecules, which can exist and replicate independently in bacterial cells. Restriction enzymes can cut the plasmid DNA into fragments. In the experiment a plasmid and three restriction enzymes *Bam*HI, *Pst*I and *Hind*III are provided. You will use the three restriction enzymes to digest the plasmid DNA and run agarose gel electrophoresis. You need to determine the restriction

enzyme sites and calculate the size of restriction fragments between cutting sites according to migration distance of DNA fragment, which is inversely correlated to the logarithm of the length of fragment.

Reagents

1. 1×TAE buffer –Tris-acetate-EDTA
2. DNA dye - GeneFinder™ containing anthocyanin and sucrose
3. *Bam*HI
4. *Pst*I
5. *Hind*III
6. Plasmid DNA
7. DNA size standard
8. Distilled water

Equipment

1. Lab gloves
2. Marker pen
3. 0.5 ml centrifuge tubes
4. Centrifuge tube holder
5. Pipettes
6. Centrifuge
7. Incubator
8. Agarose gel electrophoresis apparatus

9. Fluorescence gel imaging systems (use it with lab assistants),

Procedure and operation of equipments

1. **Using a Pipette**:

A 0-10µl pipette is provided for the experiment. The volume is adjusted by turning the setting ring. The digits of the volume display should be read from top to bottom. After attaching an appropriate tip, press the control button down to the first stop and insert the tip into the liquid. Slowly release the button until it reaches a complete stop to aspirate (suck up) the sample. Then, insert the tip with the liquid to the target places (tubes or wells) and press the button down slowly to the second stop until all collected liquid is completely expelled from the tip. Eject the used tip to the trash by pressing the ejector button.

2. Using a Centrifuge

Press the stop lever down to open the lid. Load tubes into the rotor. Be sure to balance the load properly. Close and firmly press down the lid until the lid locks into its

position. The rotor will begin spinning when the lid is completely closed. Let the centrifuge run for 20 seconds. Push the stop lever, open the lid and remove the tubes after the rotor has stopped spinning.

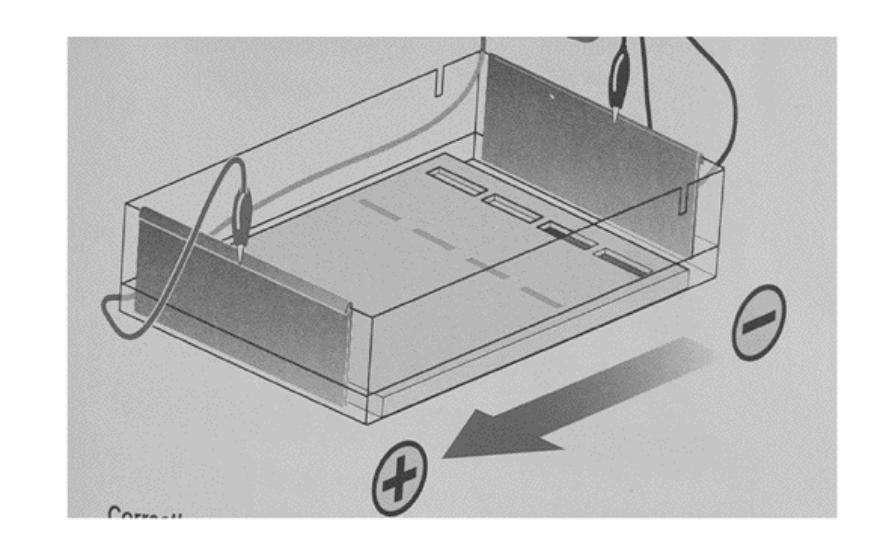
3. Using Restriction enzyme digestions

Type II restriction enzymes recognize certain DNA sequences and digest DNA at the recognition sites. The plasmid DNA provided to you should be digested by three different enzymes: *Bam*HI, *Pst*I and *Hind*III. Add the appropriate amount (1μl) of enzyme(s) to the plasmid DNA in centrifuge tube and close the lid of the tube. Mix it well by gently tapping the bottom of the centrifuge tube. Incubate the centrifuge tubes at 37°C for 15 min in the incubator.

4. Using the agarose gel electrophoresis apparatus

The 0.8% Agarose gel with wells is ready for use. Fill the electrophoresis tank with 1×TAE buffer and let the buffer cover the gel. The buffer surface should be about 3-4mm above the agarose gel surface. Load 10 μl of the sample, which contain (1) plasmid DNA cleaved with restriction enzymes and (2) DNA dye, into the wells of the gel. **Please note that** the pipette tip should be 1-2 mm above the bottom of the well so that you can load all of the samples into the wells without puncturing the bottom of the wells. After loading the samples, close the cover of the electrophoresis tank. Note that Red wire connects to the anode and Black wire connects to the cathode. Call the laboratory assistant to turn on the power supply by raising the blue card. Run

the samples at 100 volt for 40 min. After 40 minutes call the assistant to turn off the power supply by raising the blue card. Every competitor will use one electrophoresis tank, while every 2 competitors share one power supply.



5. Gel imaging system

This system is operated by lab assistants. Your samples contain a non-toxic dye that binds DNA fragments for visualization.

Experimental procedure

1. Label eight 0.5-ml centrifuge tubes 1 through 8 with a marker pen, Add solutions to each tube as follows:

Table 1. Digestion of plasmid DNA with restriction enzymes

No.	Plasmid DNA (μl)	*Bam*HI (μl)	*Pst*I (μl)	*Hind*III (μl)	ddH_2O (μl)
1	5	1			9
2	5		1		9
3	5			1	9
4	5	1	1		8
5	5	1		1	8
6	5	1	1	1	7
7	5				10

2. Mix well and incubate tubes 1-6 at 37°C for 15 minutes. Leave tube 7 in the tube holder. If you found droplets of the solution on the inside tube wall, you may used the centrifuge to spin them to the bottom of the tube. The centrifuges are provided on your table.
3. Put the agarose gel (previously prepared for you) into the electrophoresis tank, pour 1×TAE buffer into the tank and let the buffer cover the gel about 3-4mm. The gel has 10 wells for sample loading.
4. Add 6 μl DNA size standards into the No.8 centrifuge tube.
5. Add 3 μl of 5X dye to each tube and mix them well.
6. Load 5 μl of DNA size standards (tube No. 8) into the **First** well of the gel. Load all of your plasmid samples from the second well through to the eighth well in the order of Table 1. Please note that tube numbers differ from the lane numbers in which they are loaded. Use a clean tip for each load. Close the cover of the

electrophoresis tank. Call the assistant by raising the blue card to turn on the power supply. Run the samples at 100 volt for 40 min.

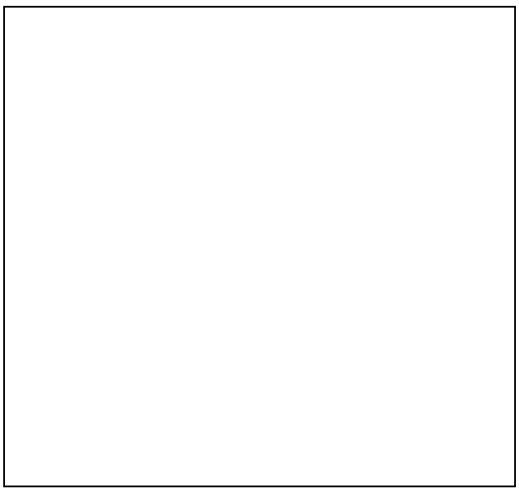
(Note: during your waiting time for completion of electrophoresis, please undertake task 2 and finish it.)

6. After the electrophoresis has run for 40 min, call the assistant to turn off the power supply by raising the blue card. Wear gloves and take out the gel holder. 8. Put your gel into the box with your competitor's number. Close the lid and leave the box on the table. A lab assistant will take the gel image and print a copy for you.

Separation of plasmid DNA restriction fragments with agarose gel electrophoresis (24 points: 3 points for each lane). The score for this task will be given by a professor in charge of this test.

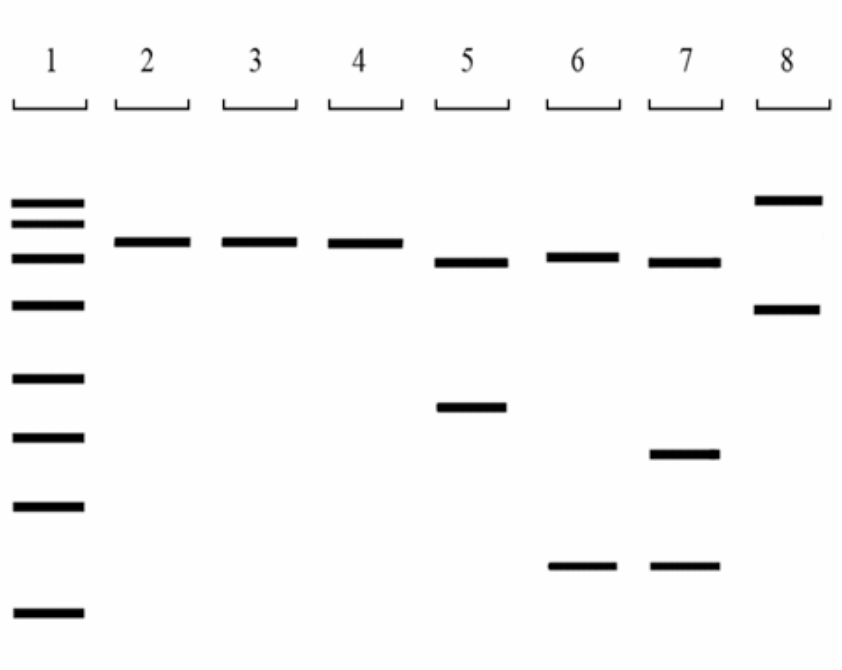
Three points for each lane: No DNA, no point; smearing lane with clear bands, minus 1 point; incomplete digestion, minus 1 point; faint bands, minus 1 point.

Your gel image will be posted below once it is printed by the lab assistant.



Task 2: Determination of restriction enzyme sites and DNA fragment size of restriction fragments. (16 points)

Due to time limitation, you will not be able to run your own gel for size analysis. However, the figure below is an agarose gel profile of DNA fragments, in which an identical plasmid was digested with the same three DNA restriction enzymes. The procedure for digestion and loading positions of each digestion in the gel are identical to the instruction in task 1. Please answer the following questions according to the profile below.



Question 1. How many sites does this plasmid have for *Pst*I, *Bam*HI and *Hind*III, respectively? (3 points)

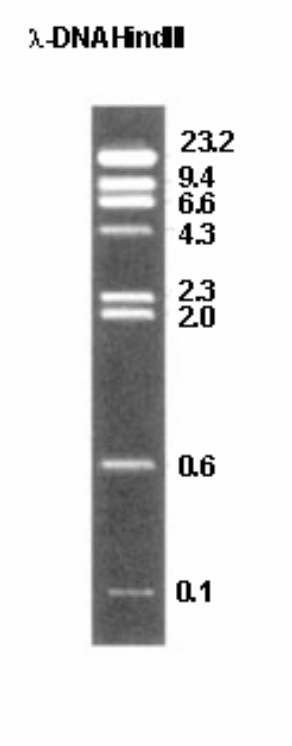
A. *Pst*I:1, *Bam*HI: 0, *Hind*III : 2.

B. *Pst*I:2, *Bam*HI : 0, *Hind*III : 2.

C. *Pst*I:2, *Bam*HI : 1, *Hind*III : 0.

D. *Pst*I:1, *Bam*HI : 1, *Hind*III : 1.

Question 2. Linear lambda DNA is often digested with restriction enzymes and used as a molecular standard in running agarose gels. The figure below is a profile of lambda viral DNA fragments obtained with *Hind*III digestion. The numbers on the right side of the gel are fragment sizes in kb.



Which of the following statements is/are true? (3 points)

(1) There are 8 sites for *Hind*III on lambda DNA.

(2) Since lambda DNA can be digested by *Hind*III, the entire molecule of lambda DNA must be double stranded.

(3) The profile shown in the figure above is likely to be a fluorescent image of a dye binding to DNA fragments.

Select which answer A to D is correct

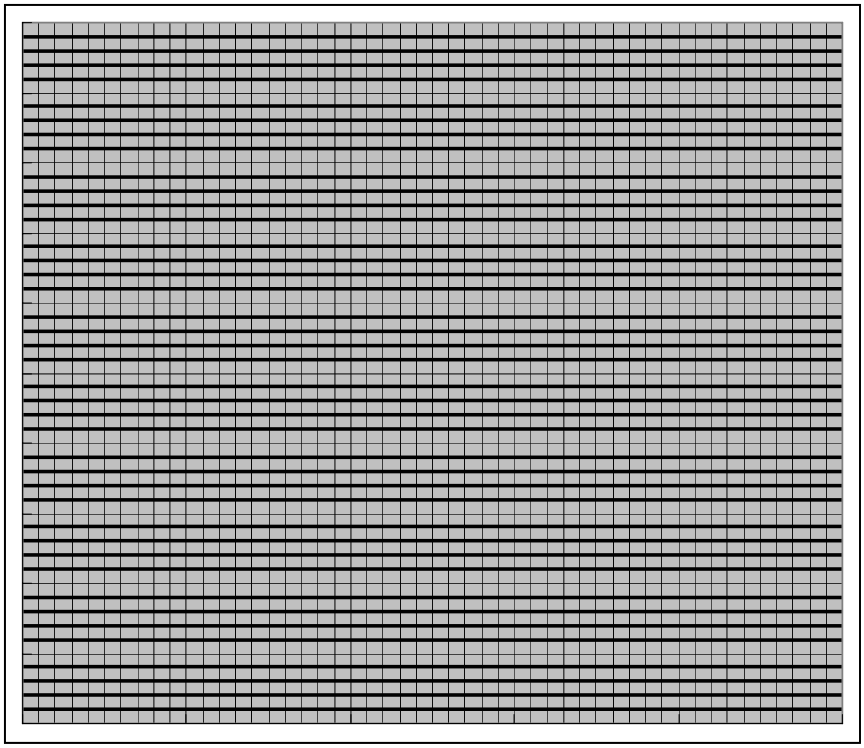
A. 1

B. 1, 2, 3

C. 2, 3

D. 3

Questions 3-5. The gel profile contains eight bands of DNA size standards in lane 1 and the sizes of the DNA fragments in lane 1 are as follows (in bp, base pairs): 200, 500, 800, 1200, 2000, 3000, 4500, 7000. It is known that migration distance of a DNA fragment is inversely correlated to the logarithm of the fragment length. Please plot the logarithm of the DNA fragment sizes (kb) versus the migration distances (cm) on the plotting (graph) paper below, and calculate the sizes (kb) of the DNA fragments.



Question 3. The size (kb) of the smaller restriction fragment between *Pst*I site &

*Hind*III site is: (3 points)

A. 2.5

B. 0.8

C. 1.1

D. 0.6

Question 4. The size (kb) of the smaller restriction fragment between *Hind*III site &

*Bam*HI site is: (3 points)

A. 0.8

B. 0.4

C. 0.5s

D. 0.6

Question 5. The plasmid length (kb) is: (4 points)

A. 5.2

B. 6.9

C. 4.8

D. 4.3

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Practical tests Part II:

Cell Biology

This part of examination contains 3 Tasks:

Task 1: Microscopes and cellular structures (15 points)

Task 2: Identification of plants with thin sections (15 points)

Task 3: Karyotype analysis (10 points)

Total Points available: 40

Total Time: 90 minutes

Task 1: Microscopes and Cellular Structures (13.5 points)

Requirement

In this task, you are provided with cell images obtained using different types of microscopy. You are required to

(1) Distinguish these cell images and choose one name for the microscopic technique for obtaining each image,

(2) Select one of the techniques for study,

(3) Distinguish between organelles in a given cell image and answer questions.

Procedure

You are supplied with two image sheets, *Image Sheet 1* and *Image Sheet 2*.

On *Image Sheet 1*, seven images (denoted 1-7) of cells or organisms are printed.

These images are obtained with the different microscopic techniques listed below:

A. Light microscopy

B. Fluorescence microscopy

C. Scanning electron microscopy

D. Ultra-thin section transmission electron microscopy

E. Immuno-electron microscopy

F. Negative staining electron microscopy

G. Freeze-fracture electron microscopy

Answer the questions according to the following descriptions.

Descriptions:

1. Image 1 is most likely to be obtained with _____. (0.9 point).

2. Image 2 is most likely to be obtained with _____. (0.9 point).

3. Image 3 is most likely to be obtained with _____. (0.9 point).

4. Image 4 is most likely to be obtained with _____. (0.9 point).

5. Image 5 is most likely to be obtained with _____. (0.9 point).

6. Image 6 is most likely to be obtained with _____. (0.9 point).

7. Image 7 is most likely to be obtained with _____. (0.9 point).

Answer the following questions about different microscopic techniques.

8. ____ is appropriate for locating specific molecules in both cells and tissues (0.9 point).

9. ____ is appropriate for visualizing details of cell and tissue surface (0.9 point).

10. ____ is appropriate for analyzing the interior of cell membranes (0.9 point).

11. ____ is appropriate for examining the fine structure of cells (0.9 point).

12. ____ is appropriate for the fine labelling (ultra structural localisation) of molecular substances in a cell (0. 9 point).

Image Sheet 2 shows the ultrastructure of a cell. Roman numbers (I-III) indicate different organelles and/or cell components.

A list of organelles and/or cell components is given below (A through F). Answer the following questions.

A. Lysosome

B. The Golgi apparatus

C. Mitochondrion

D. Microtubule

E. The endoplasmic reticulum

F. Plastid

13. The structure indicated by Roman number I is a _____. (0. 9 point).

14. The structure indicated by Roman number II is a _____. (0. 9 point).

15. The structure indicated by Roman number III is a _____. (0.9 point).

16. The structure indicated by Roman number IV is a _____. (0.9 point).

17. The cell shown in *Image Sheet 2* is likely to be a cell of _____. (choose one from below) (0.6 point).

A. Plant

B. Animal

C. Fungus

D. Eubacterium

E. Archeon

Task 2: Determination of plant types with thin sections of plant leaves (15 points)

Materials, tools and instrument

(1) Five (No.1-No.5) Petri dishes, each of which contains some leaf samples.

(2) A microscope with objective lens of 10x, 20x, 40x.

(3) Forceps, razor blade, test tube rack, slide, slide cover slip, filter paper.

Background

There are three major types of photosynthesis metabolism in the plants, called C3 metabolism, C4 metabolism and crassulacean acid metabolism. You are now required to determine which plants are C3 plants and which plants are C4 plants. The difference between them is that CO_2 fixation and sugar synthesis are performed in different cells in these two types of plants. The different structures of the leaves between C3 and C4 plants lead to different metabolism.

Task

There are five Petri dishes on the table. Each Petri dish contains pieces of leaves from a different plant. You are required to determine if the leaves are from C3 plants or C4 plants.

Procedure

Please follow the procedure below:

(1) Pick up one sample from each dish and make a thin section.

(2) Use several drops of water to wash the section off the blade onto the slide.

(3) Remove the excess water with a piece of filter paper, but keep the water around the sample.

(4) Put the cover slip onto the sample, remove excessive water and observe the specimen under the microscope.

Answer the following questions.

18. The leaves in Petri dish 1 are (3 points)

A. C3 type.

B. C4 type.

19. The leaves in Petri dish 2 are (3 points)

A. C3 type.

B. C4 type.

20. The leaves in Petri dish 3 are (3 points)

A. C3 type.

B. C4 type.

21. The leaves in Petri dish 4 are (3 points)

A. C3 type.

B. C4 type.

22. The leaves in Petri dish 5 are (3 points)

A. C3 type.

B. C4 type.

Task 3. Karyotype analysis (10 points)

Requirement:

In this task, you are asked to perform karyotype analysis. The materials are root tips from a plant. You will need to use a microscope to observe the cells of the root meristem tissue and find those cells in mitosis.

Materials, instruments and tools

(1) Root tips (approximately 5-10 mm in length) in a 1.5 ml centrifuge tube.

(2) A microscope with objective lens of 10x, 20x, 40x.

(3) A Carbol Fuchsin (a dye) solution. **(It is in a 1.5-ml centrifuge tube, labelled as CF)**

(4) Forceps, razor blade, test tube rack, slide, slide cover, filter paper.

(5) A 1.5 ml centrifuge tube containing approximately 1 ml 1 N (normal) HCl solution.

Important:

You will use 1 N HCl to treat the root tips. HCl solution is very harmful to your eyes and skin. Wear gloves and protective goggles when using HCl solution. If HCl solution comes in contact with any part of your body, please report it immediately to any instructor in the exam room.

Procedure:

You are provided with three root tips of a plant. The following procedure should be followed so that you can make an appropriate specimen to observe the chromosomes from cells in mitosis.

(1) Use the forceps to put one or two root tips into the small bottle containing 1 N HCl.

(2) Put the bottle into the water bath, which has been adjusted to 60°C, for 8 min. Note, your laboratory has several water baths with temperature adjusted to 60°C. The water baths are on the instructor's desk.

(3) Very carefully take the root tips out of the HCl solution with a forceps and put them into the beaker provided containing distilled water. Gently shake it for 1 min.

(4) Take the root tips out of the distilled water. Important: the root tips are now very fragile. It is recommended that you use the forceps to pick the root tips and don't touch the tips of the roots.

(5) Put one root tip on a slide. Cut the tissue of the root tip that is rich in dividing cells. This region is within 1 mm from the root tip. Discard other parts of the root.

(6) Put one drop of Carbol Fuchsin solution onto the root tissue you have just cut off and leave it to stain for 7 min. Squash the tissue gently with forceps so that the tissue is dispersed.

(7) Cover the dispersed tissue with a slide cover slip. Push the slide cover slip gently with a pencil or forceps until the tissue is completely dispersed and separated.

(8) Put the slide between two pieces of filter paper and put it on a flat surface. Gently press the upper filter paper down so that the tissue is further squashed. In the meantime, extra dye solution is also removed and absorbed by the filter paper.

(9) Observe your slide specimen under the microscope. Note, you might need to use all objective lenses.

Note: You are provided with three root tips to prepare your specimen. If you fail to make a good specimen for your observation, please repeat the procedure and make another preparation. However, the time for your experiment is limited.

Answer the following question:

23. How many pairs of chromosomes are there in the cells (in metaphase) from this plant? (6 points)

A. 3

B. 4

C. 5

D. 6

E. 7

F. 8

G. 9

24. If you found that different metaphase cells had different number of chromosomes, how do you determine the exact number of chromosomes? (2 points)

A. Count the chromosome numbers from several cells and use the average number as the chromosome number.

B. Count the chromosome numbers from several cells; the maximum chromosome number of a cell is the chromosome number of the plant.

C. Count the chromosome numbers of several cells in metaphase; the chromosome of the plant is the number with highest frequency.

~~25. The purpose of the treatment of root with 1 HCl at 60°C for 8 min is: (2 points)~~

~~A. Stimulate cells so that you can observe more cells in metaphase.~~

~~B. Dissolve cellulose of cell walls so that the cells are easily separated.~~

~~C. Remove ions of the cell wall so that the cells are separated.~~

~~D. Dissolve the hemicellulose of cell walls so that the cells are easily separated.~~

~~E. Puncture some tiny holes on plasma membrane so that Carbol Fuschin could penetrate into the cell.~~

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Practical Exam Part-III

Animal anatomy and ecology

This part contains three tasks:

Task 1. Determination of the distribution pattern and estimation of population size. (16 Points)

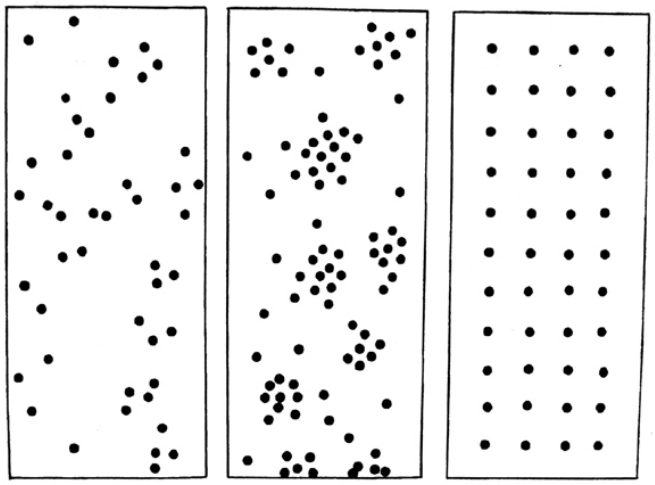
Task 2. Classification of insects. (9.8 points)

Task 3. Shrimp anatomy (14.2 points)

Task 1: Determination of distribution pattern and population size (16 points)

Introduction

Tenebrio molitor is an insect and belongs to Coleoptera. It lives in places used for food storage such as barns. The majority of the life span of *T. molitor* is in its larval stage and its adult stage is quite short. In this experiment, you will study two ecological aspects of *T. molitor*: population distribution pattern and population size. The distribution pattern of a population describes the spatial relationship of individuals of the population. It is also useful in establishing a reliable sampling method for the populations. Generally speaking, there are three types of distribution patterns: random distribution, uniform distribution and aggregated (clumped) distribution (see the associated figure)



If you divide an area into smaller identical squares and count individuals in each square, you will be able to distinguish the distribution patterns. If the distribution pattern is uniform, the square deviation (S^2) of your sampling will be zero. If the distribution pattern is random, you will get a typical Poisson (normal) distribution in your sampling. If the distribution pattern is aggregated, you will not be able to obtain a Poisson distribution in your sampling. Thus, it is possible to distinguish the three distribution patterns according to square deviation (S^2) and averages of your sampling (m).

If $S^2/m=0$，It is uniform distribution；

If $S^2/m=1$，it is random distribution；

If $S^2/m>1$，It is aggregated distribution。

Here, $m = (X_1+X_2+\ldots+X_n)/n$

$S^2=[(X_1-m)^2+(X_2-m)^2+\ldots+(X_n-m)^2]/(n-1)$

X_1，X_2，…X_n represent the number of individuals in the square 1, 2, …. and square n, respectively, and n represents total number of squares you sampled.

Materials:

A printed photograph of a tray containing some *T. molitor* is provided. The tray is divided into 7 x 7 squares.

Task: Determine the distribution pattern of *T. molitor*.

Procedure

Count the number of the larva in A1, A4, B7, C5, D2, D7, E3, F1, F6, and G3 (total number of squares is 10), and determine the distribution pattern according to the formula provided above.

Answer the following questions:

Question 1. The value of S^2/m is: (2 points)

A. 0.1

B. 0

C. 1

D. 3.4

Question 2. The distribution pattern is (2 points)

A. uniform distribution

B. random distribution

C. aggregated distribution

Question 3. Which of the following could alter the answer of question 2 above: (2 points) **(Note, there might be more than one answer)**

A. Choose the same 10 squares, but reverse the sequential order in your sampling (i.e. start from G3 and finish with A1).

B. Choose only the four corner squares (A1, A7, G1 and G7) in sampling and calculate S^2 and m to determine the distribution pattern.

C. Choose only the central five squares (D3, D4, D5, C4 and E4) in sampling and calculate S^2 and m to determine the distribution pattern.

D. Redo the sampling by choosing 10 squares randomly and calculate S^2 and m to determine the distribution pattern.

Question 4. Which of the following descriptions about the relationship between population distribution pattern and individuals of the population is accurate? (2 points)

A. Repulsion among individuals of a population would lead to uniform distribution.

B. Repulsion among individuals of a population would lead to random distribution.

C. Attraction among individuals of a population would lead to uniform distribution.

D. When the position of each individual is independent of other individuals, it would lead to aggregated distribution.

E. When the position of each individual is independent of other individuals, it would lead to uniform distribution.

The following is to estimate the population size

Population size is one of the most important factors in population ecology. A very useful tool to estimate population size is Capture-Mark-Recapture method. In this method, animals are trapped and captured. The captured animals are marked with tags, collars, etc, and released immediately. After a certain period of time, traps are set again to capture animals from the same population. A proportion of the marked (recaptured) animals in the second trapping is assumed equivalent to the proportion of marked animals in the total population. The population size (N) can be estimated by the following equation:

$$N=M\times R/P$$

Where M is the number of individuals marked during the first capture, R is the

number of individuals in second capture, P is the number of individuals in second capture that are marked.

In the population of *T. molitor*, 100 individuals are marked with black dots near their tails. These marked *T. molitor* were first released and mixed with other individuals of the population. A second capture was performed and the result is shown in the printed photograph provided.

Question 5. The population size of the *T. molitor* is: (3 points)

A. 550

B. 600

C. 610

D. 627

Question 6. In Capture-Mark-Recapture method, it is assumed that the ratios of M/N and P/R are identical. Which of the following is/are required to assure an accurate estimation of population size? (3 points) **Note, there might be more than one correct answer.**

A. The marking method should not alter the animal's normal activity.

B. Immigration occurs regularly.

C. No birth and no death during the experimental period.

D. The population should have a uniform distribution.

E. The marks on the organism should last longer than the experimental time.

Question 7. If after the experiment additional information is obtained that 40 individuals died and 30 individuals moved in between marking and recapture The new estimated population size would be (2 points) .

A. Equal to what you obtained in question 5.

B. Equal to or smaller than what you obtained in question 5.

C. Equal to or larger than what you obtained in question 5.

Task 2. Classification of insects. (9.8 points)

Instruction

There are seven specimens of beetles in the tray on your table. You are required to name each of them according to the key that follows. You will need to use a stereoscope, forceps and needle. **Note, damage to the specimen will lead to subtraction of points from your final score of practical test.**

A. ***Opatrum subaratum*** Faldermann

B. ***Blaps femoralis femoralis*** Fischer-Waldheim

C. ***Coccinella septempunctata*** Linnaeum

D. ***Potosia brevitarsis*** **(Lewis)**

E. ***Popillia quadriguttata*** **(Fairmaire)**

F. ***Polyzonus fasciatus*** **(Fairmaire)**

G. ***Chrysochus chinensis*** **Baly**

Question 8. Fill in the table below according to your classification result and mark them on your answer sheet: (1.4 x 7 = 9.8 points)

Beetle	Answer A-G
①	
②	
③	
④	
⑤	
⑥	
⑦	

Key to 7 species of beetles

1 Tarsus of fore legs, middle legs and hind legs have 5-5-4 segments.....**2**

Tarsus of foreleg, middle legs and hind legs have 5-5-5 or 4-4-4 segments….....**3**

2 Body size small and flat; there is a triangular notch at anterior edge of the labrum; wing tip at end of wing case invisible..... ***Opatrum subaratum*** Faldermann

Body size large and elevated; straight at anterior edge of the labrum; wing tip visible at end of wing case in male individual ..... ***Blaps femoralis femoralis*** Fischer-Waldheim

3 Tarsus have 4-4-4; body segments; there are 7 black round dots on the wing cases……..***Coccinella septempunctata*** Linnaeum

Tarsus segments are type 5-5-5; body not semicircular……..….. **4**

4 3rd through 8th antennal segments are branchial (gill-like) ……..**5**

Antennal segments threadlike…….…… **6**

5 There is a notch at base of each wing case; there are many white and downy (cottony) dots in shapes of strips, clouds, or waves on the pronotum and wing cases. ……..… ***Potosia brevitarsis*** **(Lewis)**

There is no notch at base of wing cases; no downy dots on the pronotum and wing cases……..…….……***Popillia quadriguttata*** **(Fairmaire)**

6 Body elongate and cylinder-like; compound eyes are reniform (kidney-shaped); antenna at frontal processes;

there are 2 yellowish transverse strips on each wing case……***Polyzonus fasciatus*** **(Fairmaire)**

Body thickset and oval; round compound eyes; body color deep green, blue, glaucous (waxy blue grey) or indigo; no transverse strips on wing cases……..…***Chrysochus***

***chinensis* Baly**

Task 3. Anatomy of a shrimp (14.2 points)

Introduction

Shrimps belong to the Crustacea in the Arthropoda. They have heteronomous segmentation. The shrimp provided for your exam has a body of 21 segments with an exoskeleton and jointed appendages.

Materials and instruments

1. One shrimp. **Note:** you only have one shrimp.
2. Stereoscope
3. Scissors, needle, forceps, insect needles, operational knife.
4. Wax tray

Experiment

Experiment contains two parts: external anatomy of the shrimp and nervous system anatomy of the shrimp.

(1) External anatomy

Observe the shrimp carefully and answer the following questions.

Question 9. How many pairs of appendages are there in the shrimp's head, thorax and abdomen, respectively? (2 points)

A. 2, 4, 10

B. 5, 8, 6

C. 4, 5, 8

D. 3, 6, 7

Question 10. Find the mouthparts of the shrimp and separate the appendages that form the mouthparts.

How many pairs of appendages is/are the mouthparts composed of? (2 points)

A. 1

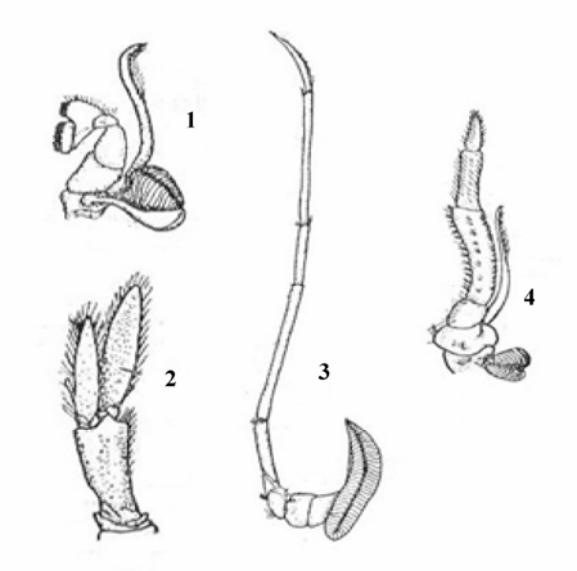
B. 2

C. 3

D. 4

E. 5

Questions 11-12. Observe the schematic structures of appendages in the figure below.



Question 11. Could you find all of these appendages on the shrimp provided to you? (2 points)

A. Yes

B. No

Question 12. Sequentially from appendage 1 through appendage 4 shown in the figure, the main functions of these appendages are: (2 points)

A. 1: Walking, 2: swimming, 3: sensing and holding, 4: sensing and holding

B. 1: Swimming, 2: sensing and holding, 3: swimming, 4: sensing and holding

C. 1: sensing and holding, 2: swimming, 3: walking, 4: sensing and holding

D. 1: sensing and holding, 2: sensing and holding, 3: swimming, 4: walking

Anatomy of the nervous system of the shrimp

Dissect the shrimp and locate the nerve cord. Answer the following questions.

Question 13. The nerve cord of the shrimp is located at: (2 points)

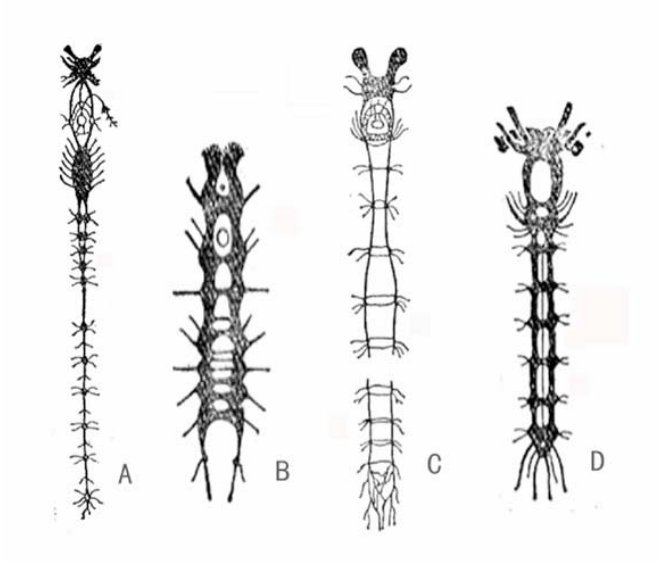
A. Dorsal side of the anterior of the body.

B. Ventral side of the posterior of the body.

C. Ventral side of the whole body of the shrimp.

D. Dorsal side of the whole body of the shrimp.

Question 14. There are 4 types of nervous systems schematically shown in the figure below.



Which nervous system shown above is identical to the nervous system of the shrimp you observed? (4.2 points)

A. Nervous system A.

B. Nervous system B.

C. Nervous system C.

D. Nervous system D.

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Country _____	Competitor# __ __ __

16th International Biology Olympiad

Beijing

July, 2005

Practical Examination

Part IV

Total time available: 90 minutes

The 16th IBO Practical Tests

First name:

Last name

Country:

Code:

Important:

1. Write your name and code on both the task paper and the answer paper sheets.
2. Make sure that all the results are written on the answer paper unless otherwise instructed.
3. There are 4 parts in the practical test. Each part lasts 90 min. You should start your **first** test according to last digit of your competitor code. For example, if you have a code of 221, your first practical test will be part I, if you have a code of 223, your first practical test will be part III.
4. Your **second** practical test is as follows: competitors from part I and part II exchange labs; competitors from part III and part IV exchange labs;
5. You go to your **third** practical test according to the following rules:

   If the last digit of your competitor code is 1, you go to practical test part III.

   If the last digit of your competitor code is 2, you go to practical test part IV.

   If the last digit of your competitor code is 3, you go to practical test part I.

   If the last digit of your competitor code is 4, you go to practical test part II.

   You should follow the instructions from your guides when switching labs.

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Practical Test, Part IV

Plant Biology

Task 1. Plant anatomy and physiology (20 points)

Materials and tools

You are provided with a set of tools and experimental materials. You will need to use other tools and instruments, including a stereoscope (stereomicroscope), microscope, Petri dishes, forceps, slides, slide cover slips, and filter paper.

You are provided with a Petri dish containing an aquatic plant.

Finish the following tasks.

(1) Initially, observe the plant with a stereoscope and answer questions 1, 2 and 3.

(2) Take one plant and put it on a slide; cut some roots off and put them on another slide and cover the slide with a cover slip. Press the cover slip slightly and observe the slide under the microscope. Answer questions 4 and 5.

(3) Take one plant and put it on a slide. Cut a leaf and put it on another slide. Cover it with a slide cover slip and press it gently. Observe the specimen you made and answer questions 6 through 8.

Questions 1-3 are about external description of the plant.

1. The stem of the plant is: (2 points)

   A. Vertical

   B. Horizontal

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C. Rosette (shortened stem)

D. Absent

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2. Which one of the following descriptions about its root is correct? (2 points)

A. It contains chlorophyll

B. It is an adventitious root

C. It is a rhizoid

D. It is a spindle-shaped root.

3. Which of the following descriptions of its leaves is/are correct? (2 points)

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(1) The leaves don't have petioles.

(2) Their leaves are bipinnate.

(3) Some leaves don't have chlorophyll.

(4) There are needle-shaped leaves.

A. 1, 2, 3, 4

B. 1, 2

C. 1, 3

D. 2, 4

E. 1, 2, 3

Questions 4-5

4. Which of the following is correct? (2 points)

A. This plant is a vascular plant

B. This plant contains vessel elements

C. This plant is a bryophyte based on its root structure.

D. None of the above

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5. A researcher grew the plant for many generations and found that no seeds were produced. Which of the following could be true based on your observation? (2 points)

(1) The researcher could have missed the seeds produced.

(2) This plant is a seedless plant.

(3) This plant does not have sexual reproduction.

A. 1, 2, 3

B. 1, 3

C. 1, 2

D. 2,

E. 3

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Questions 6-8.

6. Besides the plant tissues and cells, you should be able to observe some other immobile cells. Which of the following descriptions is/are correct about these cells? (2 points)

(1) They are unicellular.

(2) They are mostly short non-branched filaments.

(3) Some of them are branched.

(4) Their nuclei are easily observed.

A. 1,

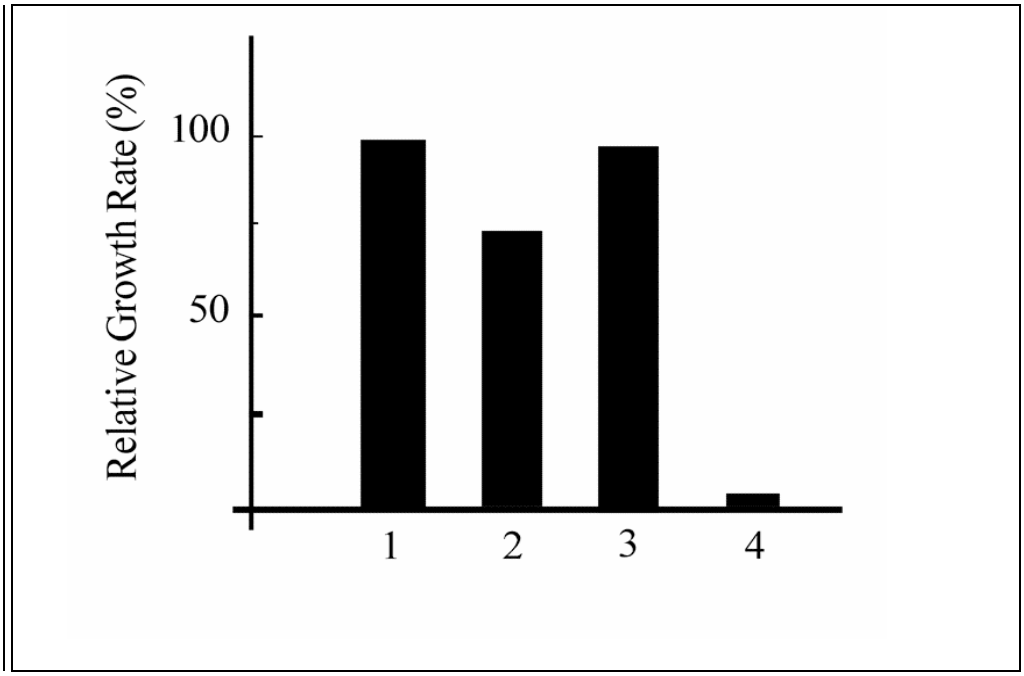
B. 1, 2, 3, 4

C. 2, 3

D. 2, 3, 4

E. 2

7. A researcher grew the plants under different conditions and obtained results shown in the figure below.



Condition 1, grown with medium A containing combined nitrogen (nitrate). The growth rate under this condition was used as 100% growth.

Condition 2, grown with medium A without combined nitrogen.

Condition 3, grown with medium A containing combined nitrogen. Ampicillin was added to a concentration of 5 μM.

Condition 4, grown with medium A without combined nitrogen. Ampicillin was added to a concentration of 5 μM.

Note, medium A is the standard medium for this plant.

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Which of the following statements is/are correct based on the results shown above? (4 points)

(1) Ampicillin is inhibitory to plant growth only under nitrogen limiting condition.

(2) The plant can grow without combined nitrogen.

(3) The root system of this plant could fix nitrogen.

(4) There are at least some microorganisms associated with the plant and they can fix nitrogen.

(5) Nitrogenase activity is directly inhibited by Ampicillin.

A. 1, 3, 5

B. 1, 5

C. 2,

D. 1, 2, 4

E. 4, 5

8. If you would like to obtain a culture of the plant that does not contain any associated organisms, what is the condition to achieve it? (4 points)

A. Grow it with combined nitrogen plus some ampicillin.

B. Grow it with combined nitrogen.

C. Grow it without combined nitrogen.

D. Grow it without combined nitrogen plus ampicillin.

Task 2 Plant pigment characterization (20 points)

Materials and tools

You are provided with 6 tubes of pigments, labelled pigment I to VI. You are also provided with a colorless solution in another tube labelled as control. You will need to use the following instruments:

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Adjustable Spectrophotometer; Cuvette cells; Adjustable pipettes; Filter paper;

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Perform the following tasks:

a. Examine the absorption spectra shown in the figure below. The five absorption spectra are obtained from different organisms and the pigment names are given in the figure. The major absorptions of these spectra are given. Among the five pigments in the figure, phycocyanin and phycoerythrin are water-soluble; chlorophyll and carotene are soluble in organic solvents; chlorophyll-protein complexes are soluble in aqueous solution when treated with detergent.

b. Use the adjustable pipette to transfer 1 ml of each pigment solution to cuvette cells. Measure the absorptions at the wavelengths in the table below. Record the results of your measurements in the table.

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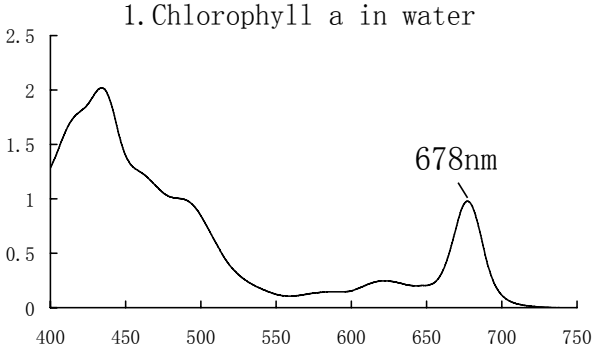
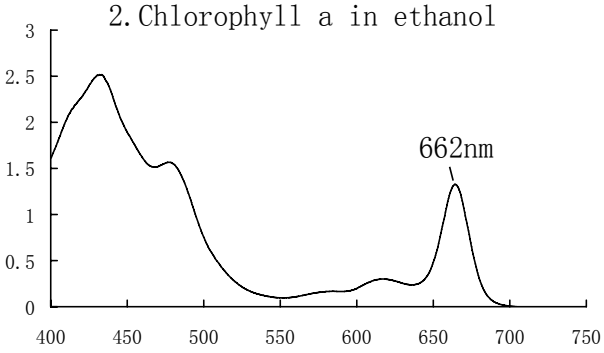
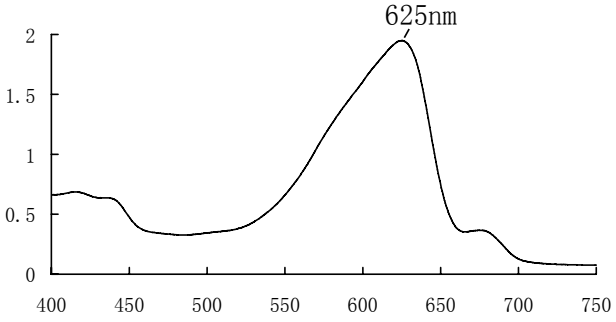
1.Chlorophyll a in water

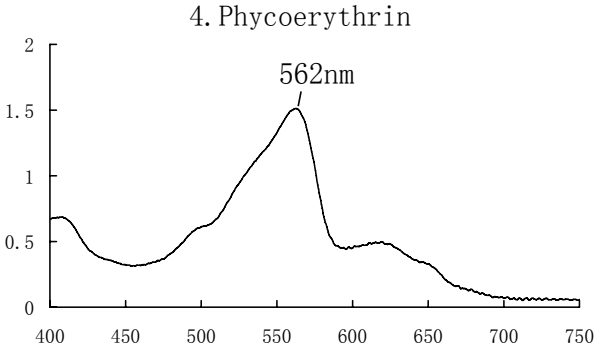


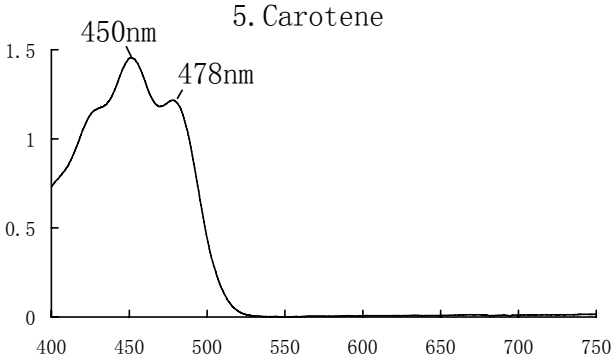
2.Chlorophyll a in ethanol



3.Phycocyanin







Solution	450nm	562nm	595nm	625nm	662nm	678nm
I						
II						
III						
IV						
V						
VI						

Answer the following questions:

Question 9. Which of the pigments would be most efficient at absorbing red light? (2 points)

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A. Phycocyanin

B. Phycoerythrin

C. Carotene

D. Chlorophyll

Question 10. Phycocyanin solution is: (2 points)

A. Solution I.

B. Solution II.

C. Solution III.

D. Solution IV.

E. Solution V.

F. Solution VI.

G. None

Question 11. Phycoerythrin solution is: (2 points)

A. Solution I.

B. Solution II.

C. Solution III.

D. Solution IV.

E. Solution V.

F. Solution VI.

G. None.

Question 12. Chlorophyll solution (in ethanol) is: (2 points)

A. Solution I.

B. Solution II.

C. Solution III.

D. Solution IV.

E. Solution V.

F. Solution VI.

G. None.

Question 13. Carotene solution is: (2 points)

A. Solution I.

B. Solution II.

C. Solution III.

D. Solution IV.

E. Solution V.

F. Solution VI.

G. None.

Question 14. Protein-Chlorophyll complex in detergent-treated solution is: (2 points)

A. Solution I.

B. Solution II.

C. Solution III.

D. Solution IV.

E. Solution V.

F. Solution VI.

G. None.

Question 15. Which of the following pigments (1, 2, 3, or 4)is/are present in all algae and higher plants? (2 points)

(1) Chlorophyll

(2) Carotene

(3) Phycoerythrin

(4) Phycocyanin

A. 1, 2, 3, 4

B. 1, 3, 4

C. 1,

D. 1, 4

E. 1, 2

Question 16. A cyanobacterium contains chlorophyll, carotenoids and phycocyanin as major pigments. When a culture of the cyanobacterium is extracted with 80% acetone and centrifuged, what color do you expect to see in the pellet? (3 points)

A. Orange

B. Blue

C. Green

D. Purple

E. Colorless

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Question 17. In the analysis of proteins with isoelectric focusing (IEF) gel electrophoresis, one often uses coloured proteins with known pI (isoelectric point) values as pI standards. Among these proteins are phycocyanin and phycoerythrin. No chlorophyll proteins are used as IEF gel standard. Which of the following is the reason why no chlorophyll-proteins are used as IEF gel pI standard? (3 points)

A. Green colour is not visible in the IEF gel.

B. Chlorophyll molecules are too small to be focused.

C. It is often difficult to obtain enough materials of chlorophyll-proteins from plants.

D. Chlorophyll molecules are not covalently attached to proteins.