The Experimental Tests

1. Animal and Plant Anatomy (100 points, 90 minutes)

Dear Participants,

- In this test, you have been given the following 2 tasks:
  Task 1: Animal anatomy (50 points)
  Task 2: Plant anatomy (50 points)
- **You must 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 and equipment listed for each task. If any of these items are missing, please raise your hand.
- At the end of the test, put the Answer Sheet and Question Paper in the envelope. The supervisor will collect this envelope.

Good Luck!!

Task 1. Animal Anatomy (50 points)

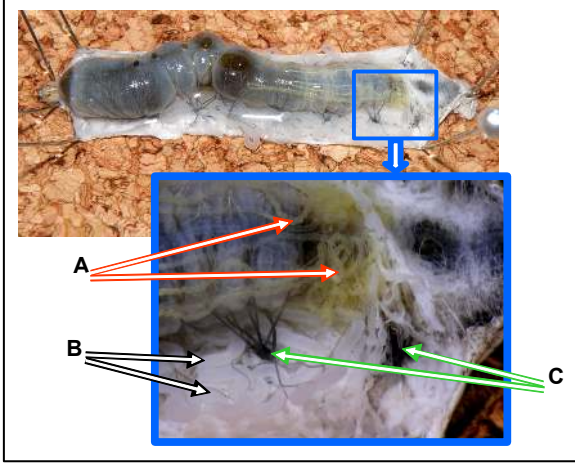
Materials and Equipment	**Quantity**
1. Vessel containing two caterpillars anesthetized	1
2. Vessel containing one caterpillar non-anesthetized	1
3. Dissecting plate	1
4. Forceps	2
5. Scissors	1
6. Disposable pipette	1
7. Dissecting needle equipped with holder	2
8. Dissecting pins	20
9. Compound binocular microscope (equipped with illuminator)	1
10. Set of color pencils: one “O” (orange), one “B” (blue), and one “G” (green)	1
11. Photo of a dissected caterpillar (included in your envelope)	1
12. A Petri dish for discarding dissected larva	1

Introduction

Even in insects which undergo complete metamorphosis, the body structure of the adult and larva are basically common. After closely observing a non-anesthetized caterpillar and dissecting and closely observing anesthetized caterpillars or moth (*Bombyx mori* Linné) larvae (silk worm), answer the following questions. When you dissect the caterpillars, do it in the dissecting plate filled with water, using suitable equipments such as forceps, scissors, dissecting needle with holder, dissecting pins.

Task 1

Photo of dissected caterpillar, dorsal view



Q.1.1. (1 point×2 = 2 points) The insect body is composed of three regions, the head, thorax and abdomen. Show the boundary

between the head and thorax by drawing an orange line with orange color pencil “O” and the boundary between the thorax and abdomen by drawing a blue line with blue color pencil “B” on the photo of the caterpillar in the Answer Sheet.

Q.1.2. (3 points) On each side of the caterpillar’s head, you will find an eye patch. How many small eyes are in the eye patch of one side of the caterpillar head in front of you? Answer using numerals.

Q.1.3. (3 points) Insects breathe by means of a tracheal system, with external openings called spiracles. How many pairs of spiracles do the caterpillars in front of you have? Answer using numerals.

Q.1.4. (6 points +[2+2]×3 points = 18 points) The photo in your envelope shows a dorsal view of a dissected caterpillar. Dissect an anesthetized caterpillar by yourself **exactly as shown in photo**. (You may use the second caterpillar if required) When you have finished the dissection, call your assistant by raising your hand. Your assistant will take a photograph of your specimen for evaluation **(6 points)**. *You should check the photograph of your dissected specimen after it has been taken*.

Closely observe the internal structures of the caterpillar, focusing on where the tubular structures A, B and C arise. Answer the name and function of each of the tubular structures A, B and C by choosing the appropriate answer for the name from numerals 1-10 and function from the alphabet a-j.

Names 1: salivary gland; 2: oviduct; 3: malpighian tubule; 4: appendix;
5: trachea; 6: prothoracic gland; 7: silk gland; 8: corpora allata;
9: fat body; 10: seminal duct

Functions a: secretion of juvenile hormone; b: support of digestion;
c: respiration; d: secretion of silk; e: secretion of prothoracic hormone;
f: restoration of fat; g: excretion; h: transport of egg;
i: transport of sperm; j: secretion of saliva

Q.1.5. (2 points×3 = 6 points) The insect body contains different kinds of internal organ systems. Closely observing non-anesthetized and dissected caterpillars, show the positions of the central nervous system, digestive system (gut) and circulatory system (heart), by drawing them into the image of the caterpillar prepared in the Answer Sheet using the colors as indicated below.

Central nervous system - orange color pencil “O”
Digestive system - blue one “B”
Circulatory system - green one “G”.

Notice: If you can show the positions of the systems in the image of the caterpillar, there is no need to copy their exact shapes: however, in drawing the digestive systems, you should clearly show both ends.

Q.1.6. (4 points) The central nervous system of insects is composed of the aggregations of cell bodies or the ganglia and the bundles of nerve fibers or the nerve cords connecting ganglia. How many ganglia does the dissected caterpillar have? Answer using numerals.

Q.1.7. (4 points×3 = 12 points) Show the positions of the anteriormost, anterior-second and posteriormost ganglia by drawing arrows and labeling with “A” for anteriormost, “2” for anterior – second and “P” for posteriormost with black pencil in the image of the caterpillar

used in **Q.1.5**.

Q.1.8. (2 points) How many nerve cords are there between each pair of ganglia? Answer using numerals, choosing the correct number from 1 to 4.

Task 2. Plant Anatomy (50 points)

In this task, fruit and flower morphology are examined and the developmental process is studied.

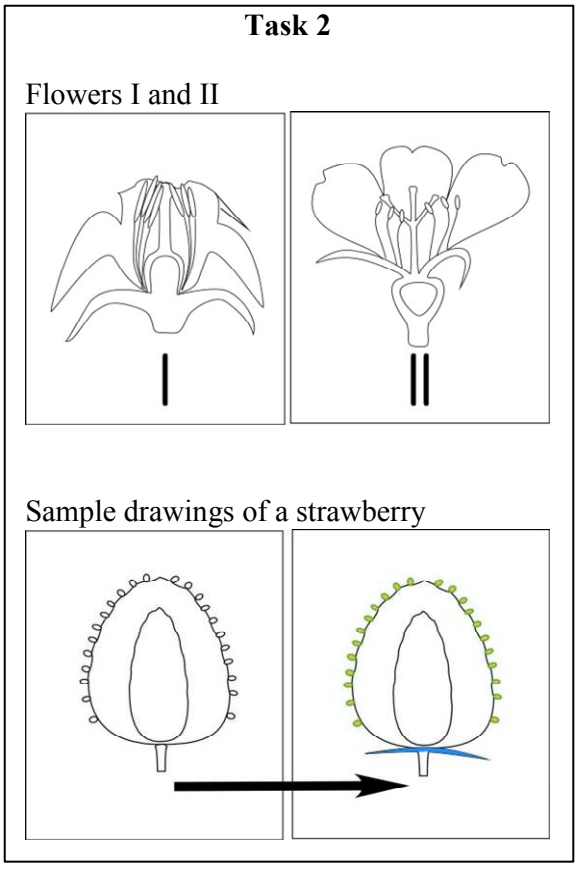
Part A. Seed morphology and reserve substances

Materials and equipment	Quantity
1. Petri dishes containing seeds labeled I to IV	4
2. Compound binocular microscope (used in Task 1)	1
3. Forceps (used in Task 1)	2
4. Knife	1
5. Scalpel	1
6. Bottles of staining and rinsing solutions (IKI, IKI-R, CBB, CBB-R, OR, OR-R)	6
7.Small Petri dishes for staining	12

Introduction

Morphology and reserve substances vary across plant species. Reserve substances can be distinguished by staining.

Q.2.A.1. (27 points) There are 4 kinds of seed (I to IV) in Petri dishes. The seeds labeled IV are *Vigna angularis*, a kind of legume which are given as an example. The seeds have been soaked for 24 hours. From some seeds, the seed coat was removed. Dissect the seeds using scalpel or knife, and stain each of them and their sections separately using all three staining solutions. Then, observe the stained seed samples including the sections of tissues under the stereomicroscope, and examine the degree of staining. Look at the samples carefully and fill the degree of staining in the Box of Q.2.A.1. in the answer sheet using the following symbols: "±" for weak staining, "+" for medium staining, "++" for strong staining. Use "-" for samples not stained, and "N" for seeds which do not have the indicated tissue.



Caution

· Some seeds are potential allergens. Wear gloves and do not touch them with your bare hands.

· Do not allow the staining solutions to contact your skin. If they touch your skin, rinse the area thoroughly with distilled water.

Staining and rinsing solutions:

Staining solution	Rinsing solution	Stain for	Color	Property
IKI	IKI-R	Starch	Purple	Aqueous solution
CBB	CBB-R	Protein	Blue	Contain ethanol and acetic acid
OR	OR-R	Lipid	Red	Contain ethanol

Staining method:

· Use small Petri dishes for staining and rinsing.
· Stain for 5 to 10 minutes in staining solution.
· Then, rinse the specimens well with rinsing solution.

Part B. Development of fruits

Materials and equipment	**Quantity**
1. Tomato fruits labeled (A)	3
2. An apple fruit labeled (B)	1
3. Drawings of flowers labeled (I and II) and strawberry fruits (included in your envelope)	1
4. Forceps (used in Task 1)	2
5. Knife	1
6. Colored pencils (orange (O), blue (B), green (G)) (used in Task 1)	3
7. White tray	1

Introduction

A fruit may develop from some part of a single flower. Therefore, the morphological features of a fruit are closely related to those of its flower.

Q.2.B.1. (4 points) There are fruits of tomato (A) and apple (B). Cut the fruits transversely and vertically on a paper towel in the white tray. Compare the fruits and flower drawings (I and II). Enter the number of the flower (I or II) that corresponds to each fruits (A, B) in the Box of Q.2.B.1. in the Answer Sheet.

Q.2.B.2. (11 points) Using a black pencil, draw and indicate ovules (or seeds), carpels (and/or tissue derived from carpel), and sepals on the vertical illustrations of the fruits (A1 and B1) of Q.2.B.2. in the Answer Sheet. Then, color the following tissues on the same fruit drawings (A1 and B1) in the colors designated. Refer to the strawberry drawings.

Ovule (or seeds): color pencil O (orange)
Carpels (and/or tissue derived from carpel): color pencil G (green)
Sepals: color pencil B (blue)

Q.2.B.3. (8 points) Complete the drawings of the transverse illustrations of the fruits (A2 and B2) of Q.2.B.3. in the Answer Sheet. Draw additional lines and color the ovules (or seeds) and carpels (and/or tissue derived from carpel) in the colors designated.

Ovule (or seeds): color pencil O (orange)
Carpels (and/or tissue derived from carpel): color pencil G (green)

**********END OF PRACTICAL TEST 1**********

2. Biochemistry (100 points, 90 minutes)

Dear Participants,

- In this test, you have been given the following 2 tasks:
  Task 1: Measurement of acid phosphatase activity (70 points)
  Task 2: Protein determination (30 points)
- **You must 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 and equipment listed for each task. If any of these items are missing, please raise your hand.
- At the end of the test, put the Answer Sheet and Question Paper in the envelope. The supervisor will collect this envelope.

Good Luck!!

How to use the spectrophotometer

1. The screen of spectrophotometer (Shimadzu UVmini-1240) must show 400 nm (Fig. 1). If not, raise your hand. ABS value shown may not be 0.000.
2. Fill a plastic semi-micro cuvette with distilled water (DW) at least up to the shoulders inside (Fig. 2)
3. Insert the cuvette into the cuvette holder of the instrument, with the transparent surfaces facing to the left and right (Fig. 3).
4. Shut the lid (Fig. 4).
5. Press 'AUTO ZERO' button (Fig. 5). By this manipulation, the instrument regards the level of absorbance by the cuvette plus water as zero. This will be used as the blank control for the rest of this experiment.
6. Now, you are ready to measure absorbance of samples.
7. Replace the water with a sample solution and read an ABS value after the lid is shut. The absorbance is caused by solutes in the sample solution.
8. You do not have to wash the cuvette after every measurement, if you measure a series of samples from the dilute to the concentrated.

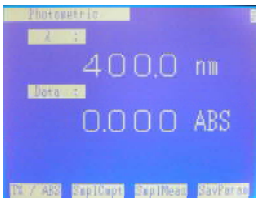


Fig. 1



Fig. 2

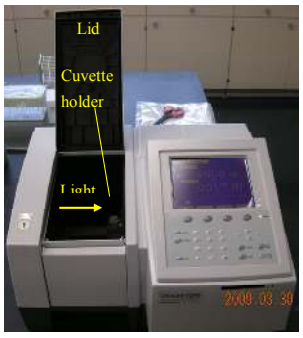


Fig. 3


Fig. 4

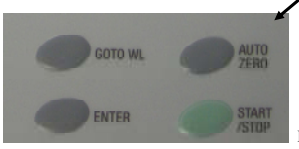


Fig. 5

Introduction

Acid phosphatase liberates phosphate ions from phosphorylated molecules under acidic conditions. The purpose of this experiment is to determine the specific activity of the acid phosphatase. You will measure activities of the acid phosphatase using a crude extract from potato in Task 1, and determine a protein concentration of the crude extract in Task 2.

Specific activity, which is the activity per unit time per unit weight of protein, is obtained from Tasks 1 and 2. Specific activity is an index of purity; it increases as the enzyme is purified.

Caution

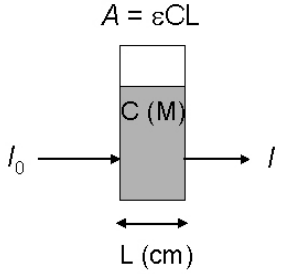
1. You will be handling small amounts of toxic substances (*p*-nitrophenol and NaOH).You can choose to wear disposable gloves and safety goggles in the experiments if you like.
2. In calculations where answers to previous questions are needed, partials marks will be given if calculated formulas are correct, even if answers are incorrect.

Materials and Equipments	**Quantity**
1. Spectrophotometer	1
2. Micropipettes (P1000)	2
3. Micropipettes (P200)	1
4. Tips (one box each for P1000 and P200)	2
5. Plastic cuvette	1
6. Test tube holder that accommodates 6-1 to 6-6	1
6-1. Crude extract of acid phosphatase (4 ml in a 15-ml plastic tube, labeled '1x enzyme')	1
6-2. 0.5 M Na acetate buffer (pH 5.6) (2 ml in a 15-ml plastic tube)	1
6-3. 5 mM pNPP (4 ml in a 15-ml plastic tube)	1
6-4. 0.5 M NaOH (8 ml in a 15-ml plastic tube)	1
6-5. 3% NaCl (10 ml in a 15-ml plastic tube)	1
6-6. Test tubes (Glass)	6

Task 1. Measurement of Acid Phosphatase Activity (70 points)

The activity of acid phosphatase is measured by an enzymatic reaction that converts p-nitrophenyl phosphate (pNPP) to *p*-nitrophenol (pNP), liberating phosphate. The product, pNP, absorbs light whose wavelength is 400 nm with an absorption coefficient* ($\varepsilon_{400\ nm}$) of 19000 M^{-1} cm^{-1} at extremely alkaline pH. Reaction mixture for an acid phosphatase is slightly acidic. Thus, it must be alkalinized for quantification of pNP. In Task 1, you will measure a time course of the reaction and obtain absorbance change per minute that is caused by 1 ml of crude extract. The absorbance change is converted to concentration change by using $\varepsilon_{400\ nm}$. Then, you will calculate a mol number of pNP molecules produced during the reaction by multiplying the concentration change by a volume of sample that is subjected to the measurement of absorbance.

＊What is an absorption coefficient？



A, absorbance
ε, absorption coefficient (M^{-1} cm^{-1})
C, concentration (M=mol $litre^{-1}$)
L, light path length (cm)
I_0, intensity of incident light
I, intensity of transmission light
Absorbance (A) is a physico-chemical property of solution that expresses to what extent a

solute absorbs light at a specific wavelength. Absorbance is in proportion to concentration (C) and light path length (L). The constant in the equation is a value characteristic to the solute, and is termed the absorption coefficient (ε). Thus, the relationship is formulated as $A=\varepsilon C$ (M=mol litre^{-1}) L (cm). Absorbance can be converted to concentration, since ε is given and L is 1 cm in this experiment. The dimension of ε is M^{-1} cm^{-1}, because absorbance is an absolute number without units.

Two enzyme concentrations are to be examined in Task 1. Find the test tube on which '1x enzyme' is labeled, which contains a crude extract of acid phosphatase. Next, find the 15-ml tube that contains 3% NaCl and remove 1ml of the solution so that the tube now contains 9 ml of 3% NaCl. Add 1 ml of the '1x enzyme' solution to it by using a micropipette, which makes '0.1x enzyme' solution. Relabel the tube as '0.1x'. Next, find 6 empty test tubes. Label each tube with an enzyme concentration and a reaction time as follows.

'0.1x', 20 min
'1x', 20 min
'0.1x', 10 min
'1x', 10 min
'0.1x', 1 min
'1x', 1 min

Q.1.1. (10 points) First, make an experimental schedule in order to perform all reactions, by describing start (○) and stop (●) signs for each reaction in the table in the Answer Sheet, allowing at least 1 min between the beginning of each reaction. An example for the reaction of '0.1x, 20 min' has been described in the table in the Answer Sheet.

Q.1.2. (15+10 points) Perform the enzymatic reactions according to the protocol described below and the schedule you made in Q.1.1. Use a new pipette tip in every manipulation. Agitate a mixture by tapping a test tube immediately after an addition. After you perform all the reactions, measure A_{400} of the samples. Write the obtained values in the table in the Answer Sheet, and plot them in the graph. **Please note that since water has been used as blank, the line will not pass through 0 (zero) on Y-axis (origin).**

Protocol for measurement of acid phosphatase activity

1) Mix 0.12 ml of 0.5 M Na acetate buffer (pH 5.6) and 0.24 ml of 5 mM pNPP in a test tube. Start the reaction by adding 0.24 ml of an enzyme solution.
2) After the reaction times of 1, 10, and 20 min, respectively, stop the reaction by adding 0.6 ml of 0.5 M NaOH. NaOH stops the reaction and converts the pNP produced into a yellow-colored (A_{400}-absorbing) form.
3) After all reactions are stopped, measure A_{400} of the samples.

Assay of potato acid phosphatase

0.5 M Na acetate buffer(pH 5.6)	0.12	ml
5 mM pNPP	0.24	ml
Enzyme	0.24	ml
0.5 M NaOH	0.6	ml
Sum	1.2	ml

Q.1.3. (15 points) Which enzyme concentration gave better linearity in the relationship between time and A_{400}? Circle the correct one on the Answer Sheet. Read the slope of this straight line from the graph.

Q.1.4. (5 points) Using the slope obtained in Q. 1.3, calculate the activity in the form of A_{400} change per min per 1 ml of an enzyme solution of concentration '1x'. The length of the light path (L) is 1cm. Your answer should be written with your calculations and the appropriate unit in the Answer Sheet.

Q.1.5. (5 points) Convert the absorbance change obtained in Q.1.4 to a concentration change by assuming the ε_{400} of pNP to be 19000 M^{-1} cm^{-1}. Your answer should be written with your calculations and the unit per min per 1 ml of '1x enzyme' solution in the Answer Sheet.

Q.1.6. (5 points) Convert the concentration change obtained in Q.1.5. to a change in number of moles of pNP. Your answer should be written with your calculations in moles per min per ml of '1x enzyme' solution in the Answer Sheet.

Q.1.7. (5 points) Calculate the total activity (in moles per min) in 4 ml of '1x enzyme' solution that was initially given.

Task 2. Protein Determination (30 points)

Protein concentration is determined by using a standard protein such as bovine serum albumin (BSA). In Task 2, you will determine a BSA-equivalent concentration of the 1x enzyme solution by the Bradford method. The Bradford method takes advantage of an increase in absorption of Coommassie Brilliant Blue at 595 nm when it is bound to protein.

By diluting a concentrated BSA solution (0.4 mg protein ml^{-1}) with 3% NaCl, a 1/2-dilution series was made (0.4, 0.2, 0.1, and 0.05 mg protein ml^{-1}). The dilution series of BSA and the 0.1x enzyme solution, which was made in Task 1, were all similarly treated with dye. Optical density at 595 nm (OD_{595}) of these samples was measured and recorded in the table below.

Table

Sample	[BSA] ($mg \cdot ml^{-1}$)	OD_{595}
	0.00	0.000
	0.05	0.070
	0.1	0.143
	0.2	0.261
	0.4	0.521
0.1x enzyme solution		0.180

Optical density (OD), a measure of the extent to which a substance transmits light or the 'absorbance' of suspension of particles.

Q.2.1.(10 points) Plot OD_{595} against BSA concentration in the graph in the Answer Sheet and depict an approximate straight line.

Q.2.2.(10 points) Estimate a protein concentration of the 0.1x enzyme solution from the graph, and obtain the protein concentration of the 1x enzyme solution.

Q.2.3.(10 points) Calculate the specific activity (activity per min per mg protein) of the 1x enzyme solution. Your answer should be written with your calculations and the unit per min per mg protein in the Answer Sheet.

**********END OF PRACTICAL TEST 2**********

3. Genetics (98 points, 90 minutes)

Dear Participants,

- This test includes the following 5 tasks:

Task 1:	Phenotypic observation of mutant flies	(9 points)
Task 2:	Inheritance of white eye mutation	(33 points)
Task 3:	Separation of eye pigments	(18 points)
Task 4:	Reading chromatography	(14 points)
Task 5:	Analysis of White Protein	(24 points)

- **You must 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 and equipment listed for each task. If any of these items are missing, please raise your hand.
- At the end of the test, put the Answer Sheet and Question Paper in the envelope. The supervisor will collect this envelope.
- This series of practicals are time consuming. You will need to be well organized and work quickly to complete the five tasks.

Good Luck!!

Task 1. Phenotypic Observation of Mutant Flies (9 points)

Materials and Equipment	**Quantity**
1. Petri dishes numbered (1)-(4) containing live fruit flies	1 set
2. Stand loupe (magnifying glass)	1

Introduction

Fruit flies are commonly used materials in genetics studies. Petri dish (1) contains the wild type, and each of the Petri dishes (2)-(4) contains different mutant flies. Observe the flies carefully by using the loupe (magnifying glass), but do not open the lid of the dishes. You may adjust the height and angle of the loupe for your observations.

Q.1.1. (9 points) In the case of each mutant, what kind of trait differs from the wild type? Choose the characteristic phenotype of the mutant trait from the following list.

A. eye color	B. eye shape	C. wing shape	D. bristle length
E. antenna shape	F. bristle shape	G. leg shape	H. proboscis shape
I. body color	J. abdomen length		

Task 2. Inheritance of White Eye Mutation (33 points)

Materials and Equipment	Quantity
1. 1.5 ml tubes containing anesthetized fruit flies labeled (5a) and (5b), (6a) and (6b), and (7)	1 set
2. Empty Petri dishes	5
3. White cardboard (place under the Petri dishes for easy observation)	1
4. Forceps	2
5. Stand loupe (magnifying glass) (used in Task 1)	1
6. 1.5 ml tube rack	1

Introduction

Wild type fruit flies (WT) have red eyes, while the mutant flies (*w*) have white eyes. *w* is a recessive mutation and located on the X chromosome. Each of tubes (5a) and (5b) or (6a) and (6b) separately contains male or female flies obtained from two different crossings. Tube (7) contains flies of both sexes from another crossing. Note that flies can be sexed by their patterns of the posterior dorsal abdomen, which is uniformly black in males.



Female



Male

Q.2.1. (8 points) Remove the flies from tubes (5a) and (5b) into different Petri dishes, and observe them by using the loupe (magnifying glass). Examine sex and eye color, and complete the table with the numbers of flies, including zero.

Q.2.2. (8 points) Remove the flies from tubes (6a) and (6b) into different Petri dishes, and observe them by using the loupe (magnifying glass). Examine sex and eye color, and complete the table with the numbers of flies, including zero.

Q.2.3. (8 points) Remove the flies from tube (7) into a Petri dish, and observe them by using the loupe (magnifying glass). Examine sex and eye color, and complete the table with the numbers of flies, including zero.

Q.2.6. (9 points) Which of the following crossings produce the flies of tubes (5a) and (5b), (6a) and (6b), and (7)? Choose all possible cases and answer with symbols.

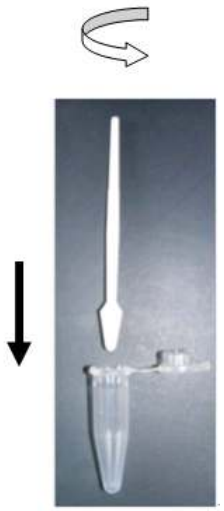
A. Homozygous red-eyed females and hemizygous red-eyed males
B. Homozygous white-eyed females and hemizygous white-eyed males
C. Homozygous red-eyed females and hemizygous white-eyed males
D. Homozygous white-eyed females and hemizygous red-eyed males
E. Heterozygous females and hemizygous red-eyed males
F. Heterozygous females and hemizygous white-eyed males

Task 3. Separation of Eye Pigments (18 points)

In addition to the materials and equipment used in Task 2, you will use the following set of equipment in this task.

Materials and Equipment	**Quantity**
1. 1.5 ml tubes (8) and (9) containing eye-pigments extraction solution	1 set (1 spare set)
2. Empty 1.5 ml tubes (10) and (11)	1 set (1 spare set)
3. Micropestles (in 15 ml tube)	2 (1 spare)
4. Centrifuge	1
5. Micropipette (P20)	1
6. Pipette tips (for P200 and P20)	1 pack
7. Empty 1.5 ml tubes (no numbers written on the lid)	2 (2 spares)
8. Cellulose/plastic sheet	1 (1 spare)
9. Micropipette (P2)	1
10. Pipette tips (P2)	1 pack
11. 50 ml tube containing solvents	1
12. Tube rack for the 50 ml tube	1

Procedure



1. Select five red-eyed and five white-eyed flies classified in Task 2 (either females or males), and remove their heads from the bodies using two pairs of forceps. ***Be sure not to crush eyes and abdomen of the flies.**
2. By using forceps transfer the heads of red-eyed flies into tube (8), the heads of white-eyed flies into tube (9), the bodies of red-eyed flies into tube (10), and the bodies of white-eyed flies into tube (11). Tubes (10) and (11) will be used in Task 5.
3. Insert a micropestle in each of tubes (8) and (9) and grind fly heads by revolving and pressing the pestle against the bottom of the tube with your hand. Use different pestles for different samples.
4. Centrifuge tubes (8) and (9) at 14,000 rpm for 3 min **(see the "Instruction for the centrifuge" at the end of this test, pages 18-19, and ask the supervisor for assistance if required)**.
5. Transfer 5 µl of supernatant from tubes (8) and (9) into new tubes.
6. Look at the cellulose/plastic sheet. The shorter sides of the cellulose/plastic sheet are the top and the bottom, and the non-glazy surface is the cellulose surface, which is used in this experiment. Write your student code with pencil at the top of the cellulose surface.
7. First, spot 1µl of the red-eyed heads extract at 1/3 from the left side and about 2 cm from the bottom of the sheet. Do not draw a line using a pencil or a marker pen, which may scratch the cellulose coating.
8. Then, spot 1µl of the white-eyed heads extract at 1/3 from the right side and about 2 cm from the bottom of the sheet.
9. When the spots dry, set the sheet into the 50 ml tube so that the bottom of the sheet touches the solvent, and close the cap tightly. Make sure the spots are not touched by the solvent. Open and close the cap of the tube quickly to minimize the leak of vapor.
10. Keep the tube straight on the tube rack to start solvent development. You can

continue with task 4 and 5 in the test and come back to this section. **Please read part 11 below before you continue**.

11. When the solvent front on the sheet reaches the 30 ml graduation mark of the tube, take the sheet out from the tube, let it dry on a piece of paper towel and close the cap of the tube. Raise your hand once the cellulose sheet is dry. (Your assistant will collect your sheet to evaluate the result.) **(18 points)**

Task 4. Reading Chromatography (14 points)

Introduction

Although some of the eye pigments involved in the compound eyes of fruit flies are invisible to our eyes, they can be visualized under UV lamp. Figure 1 shows an example of eye pigment spots resolved by chromatography and recorded under UV light. Note that the samples include not only WT (wild type) and *w* (white eyes) but also *se* (sepia eyes), *bw* (brown eyes), and *cn* (cinnabar eyes).

There are two pathways of eye pigment production in fruit flies, ommochrome pathway and pteridin pathway. The wild type eye color is formed if all pigments produced in both of the pathways are normally transferred to the compound eyes. Eyes are white if both the ommochrome and the pteridin pigments are absent. Of the pigments and their intermediate compounds involved in the two pathways, only those of the pteridin pathway can be separated by chromatography of this experiment.

The migration of each pigment during chromatography is determined by the chemical nature of the compound, the solubility of the compound to the solvent, and the migration distance of the solvent. The migration distance of a given pigment depends on the developing time of chromatography, but the Rf value is constant for each pigment, which is calculated by the following equation.

$$Rf = \frac{\text{Distance from the base line to the center of the spot}}{\text{Distance from the baseline to the solvent front}}$$

Table 1 summarizes color under UV lamp and Rf value of each pigment separated from the compound eyes of fruit flies.

Table 1. Characters of pteridin pigments in compound eyes of fruit flies

Code	Name	Color under UV lamp	Rf value
A	2-amino-4-hydroxypteridin	blue	0.57
B	biopterin	blue	0.61
C	drosopterin	orange	0.21
D	sepiapterin	yellow	0.52
E	isoxanthopterin	yellow	0.69
F	xanthopterin	green-blue	0.38
G	isosepiapterin	violet-blue	0.25

Q.4.1. (5 points) Choose the pigment from Table 1 that corresponds to each of the spots separated in the Figure 1 chromatography. Answer with the code in the table. How are the compositions of the pteridin eye pigments of the mutants different from that of the wild

type? Estimate the approximate amount of each pigment deduced from the Figure 1 chromatography. Write "++" if there is a lot more of the pigment as compared with the wild type, "+" if the pigment is present in similar amounts as in wild type, and "-" if the pigment is not present.

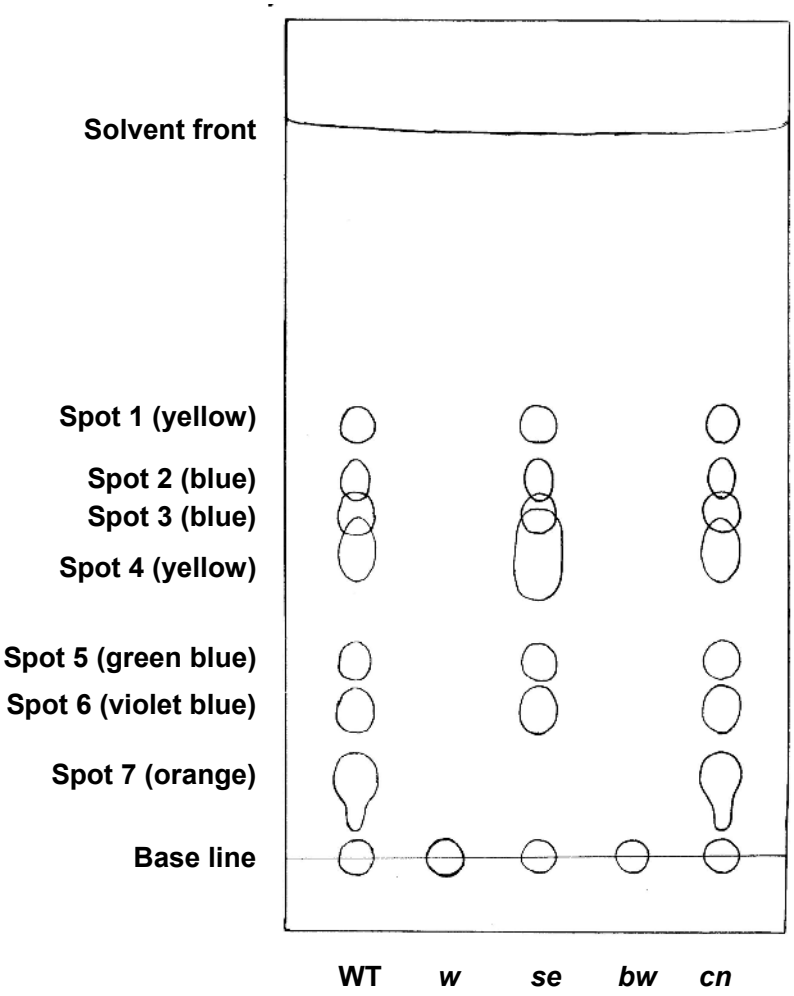


Figure 1. Chromatography of eye pigments from wild type and mutant flies

Q.4.2. (9 points) Given the eye color and the results of chromatography shown in Figure 1, which of the following abnormalities do *se* (sepia eyes), *bw* (brown eyes), and *cn* (cinnabar eyes) have? Write the corresponding alphabet.

A. Ommochrome pigments must be absent.
B. All pteridin pigments are absent but ommochrome pigments must be present.
C. Both ommochrome and pteridin pigments are absent.
D. Constituent of pteridin pigments differs from the wild type.

Task 5. Analysis of White Protein (24 points)

Materials and Equipment	**Quantity**
1. 1.5 ml tube A: Protein extraction buffer	1
2. 1.5 ml tubes (two are (10) and (11) of Task 3)	4
3. Micropestles (in 15 ml tube)	2 (1 spare)

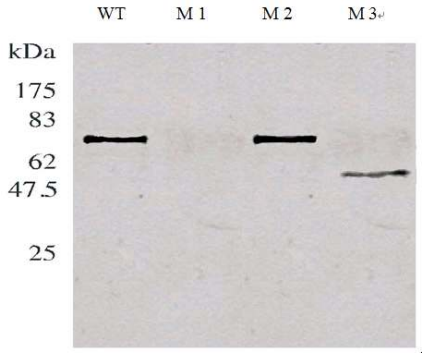
4. Electrophoresis apparatus with precast gel	1
5. Micropipetter (P200)	1
6. Micropipetter (P20)	1
7. Pipette tips (for both P200 and P20)	1 pack
8. 1.5 ml tube rack	1
9. 1.5 ml tube C: Protein electrophoresis marker	1

Protein extraction and electrophoresis

1. Add 50 µl protein extraction buffer (tube A) in the tube (10) (bodies of red-eyed flies) and (11) (bodies of white-eyed flies) prepared in Task 3. Crush the flies with the micropestle. Use different micropestles for wild type and mutant samples.
2. Centrifuge tubes (10) and (11) at 14,000 rpm for 3 min, and then transfer supernatant into fresh 1.5 ml tube **(see the "Instruction for the centrifuge" at the end of this test, pages 18-19, and ask the supervisor for assistance if required).**
3. The assistant has prepared a gel for you and it is ready for use. Load 5 µl of each sample on the slots in the middle of the gel plate in the order of molecular weight marker, red eye and white eye (from left to right). When you have finished sample loading, raise your hand for the supervisor. Your assistant will take care of the apparatus and start electrophoresis.
4. After 5 min, call your assistant by raising your hand. Your assistant will collect the lower part of the apparatus and take a photograph of the gel for evaluation **(18 points). Please check the image on the camera with your assistant.**

Analysis of protein electrophoresis data

M1, M2 and M3 flies are different mutant lines for the eye pigment genes. After separating proteins of these mutant flies through SDS polyacrylamide gel, proteins were transferred onto a nylon filter to be probed with antibody that specifically recognizes the protein encoded by the *white* gene. The following result was obtained.



Q.5.1. (3 points) Which of the following defects of eye pigment genes causes the electrophoresis results of M1, M2 and M3? Choose the corresponding symbols from A, B and C.

A. The mRNA initiation site of the *white* gene is deleted, and the gene is not expressed.
B. A stop codon mutation has occurred in the coding region of the White protein, resulting in failure of translation of carboxyl terminal peptide sequence corresponding to molecular weight 20 kDa.
C. Although a normal White protein is synthesized, genes involved in the synthesis of ommochrome pigments are defective.

Q.5.2. (3 points) Choose another defect of eye pigment gene from A, B and C that would cause the same phenotypes as M1, M2 and M3.

A. The coding sequence of the *white* gene is fused with the coding sequence of another gene by chromosomal translocation, resulting in a novel sequence encoding a fusion protein that retains antibody reacting sites but exhibits about 30 % lower molecular weight.
B. A single base substitution has occurred in the protein-coding region of the *white*

gene changing an amino acid coding sequence into another amino acid coding sequence. However, immunological reactivity of the altered protein for the antibody is not lost.

C. A large deletion exists in the chromosomal region that involves the entire *white* gene.

Instructions for the centrifuge

Ask the supervisor for assistance if required

1. Press the OPEN button at the upper-right of the operation panel (Fig. 1 - 1) to open the centrifuge lid (2).
2. The rotor is covered by a plastic cap (Fig. 2 - 3). To remove the cap, hold the cap with one hand, and unfasten the central black screw (4) anti-clockwise with the other hand.
3. There are 24 holes inside the rotor (Fig. 3). Set the sample tubes in a symmetric position, considering their balance.
4. Turn the rotor cap screw (4) clockwise to fasten the cap on the rotor.
5. Close the centrifuge lid firmly. You should hear a beep that tells complete closure.
6. The centrifuge speed (140 x 100 rotation per minute) and time (3 min) is preset. Confirm the set parameters in the windows (5) and (7) by pressing the DISP/CE button (6), and press the START button (8) to start centrifugation.
7. When centrifugation is finished, the lid (2) is automatically unlocked. Then, open the lid (2) fully and remove the rotor cap by unfastening the screw (4) anti-clockwise while holding the rotor cap with the other hand.
8. In order to not disturb the precipitates, take out the sample tubes carefully from the rotor. Leave them on the tube stand.
9. Replace the rotor cap (3) and fasten the screw (4) clockwise, and close the centrifuge lid (2).

Figure 1

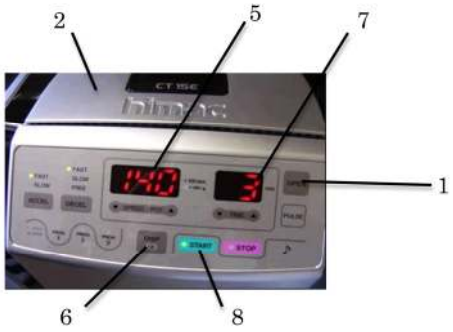


Figure 2



Figure 3



**********END OF PRACTICAL TEST 3**********

4. Cell Physiology (91 points, 90 minutes)

Dear Participants,

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

Task 1: Study on the cell cycle (61 points)
Task 2: Study on the motile mechanism of unicellular algae (30 points)

- **You must 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 and equipment listed for each task. If any of these items are missing, please raise your hand.
- At the end of the test, put the Answer Sheet and Question Paper in the envelope. The supervisor will collect this envelope.

Good Luck!!

Task 1. Study on the Cell Cycle (61 points)

Introduction

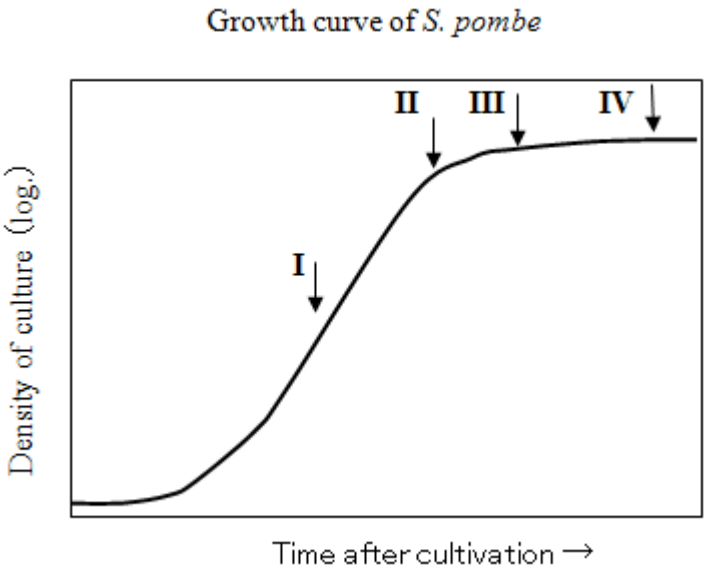
In many unicellular organisms, gene duplication and segregation occur in a controlled manner as the cell body grows. When the environmental conditions in which cells are growing become less favorable or stressful, genetic exchange is often seen via cell conjugation (mating) between cells of different mating types. That phenomenon is essential for life and is controlled by both internal and external condition of the cells. To date, we have tried to reveal these mechanisms by studying mutants in several model organisms. For example, the investigation of mutants in the fission yeast, *Schizosaccharomyces pombe* has provided us with invaluable information. Wild-type *S. pombe* cells proliferate by repeated cell elongation followed by symmetric cell division. On the other hand, under stressful conditions such as starvation, cells undergo arrested growth at an appropriate stage of the cycle, and spore formation is induced via cell conjugation to overcome the stressful conditions. The following task involves examining cell proliferation using *S. pombe*.

Materials and equipment	**Quantity**
1. Fixed culture of wild-type strain; a	1
2. Fixed culture of wild-type strain; b	1
3. Fixed culture of wild-type strain; c	1
4. Fixed culture of wild-type strain; d	1
5. Micro tube stand	1
6. Microscope	1
7. Disposable cell counter	1
8. Counter	1
9. 1.5ml microtube	3
10. Box of glass slides	1
11. Box of coverslips	1
12. Micropipette P-20 (capacity 2-20 µL)	1
13. Box containing micropipette tips	1
14. Fixed culture of wild-type strain incubated at 25°C; W25	1
15. Fixed culture of wild-type strain incubated at 36°C; W36	1
16. Fixed culture of *cdc25* mutant strain incubated at 25°C; M25	1
17. Fixed culture of *cdc25* mutant strain incubated at 36°C; M36	1
18. Photograph of cells stained with Calcofluor and DAPI	1

Part A

The growth curve of *S. pombe* wild-type haploid (n=1) incubated at 25°C is shown below.

Sampling of culture medium has been carried out at time points indicated by an arrow. Culture media a, b, c and d on the bench correspond to a sample of the culture taken at a certain time of cultivation I, II, III or IV. Observe each of the media with a microscope, and answer the following questions. Please stir the microtube just before observation.



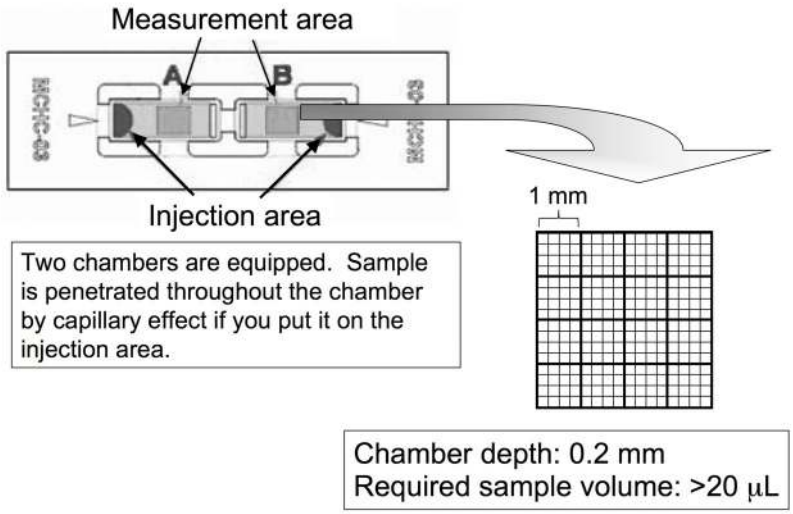
Q.1.A.1. (2x2points) Compare the cells in sample a with those in sample b, and answer the following questions.

1 In which sample are the cells rounder?

2 In which sample is there a higher population of cells undergoing cytokinesis?

Cytokinesis is defined as the part of the cell cycle from initiation of septum formation to the separation of daughter cells.

Q.1.A.2. (6 points) Measure the number of cells per 1 ml culture medium in sample a by using the cell counter as indicated below. Daughter cells that have not separated should be counted as a single cell. Write your Answer on the Answer Sheet. Notice that each student has received one cell counter but each counter has two counting chambers. You can make two measurements with this counter.



Q.1.A.3. (5 points) Measure the percentage of cells undergoing cytokinesis in the culture medium in sample a. You should count more than 100 cells in total by choosing several optical fields at random. You must write the percentage of cells undergoing cytokinesis AND the total number of cells you counted on the Answer Sheet.

Q.1.A.4. (4 points) Estimate the time period required for one round of the cell cycle of cells in logarithmic phase, provided that it takes 25 min from the beginning of cytokinesis to the separation of the daughter cells. Enter both the formula and your answer in the Answer Sheet.

Q.1.A.5. (3 points) What applies to the cells in culture medium c?

A vigorously growing
B forming spores
C conjugating
D most of cells are dead
E undergoing meiosis

Q.1.A.6. (8 points) Which culture medium (I, II, III, or IV) corresponds to a, b, c and d, respectively?

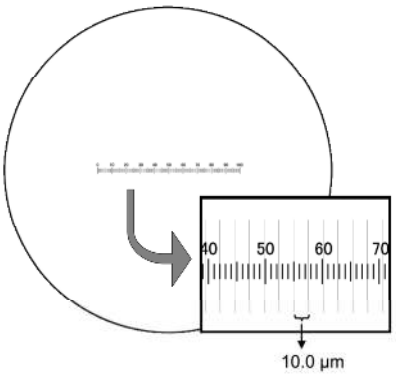
Part B

Both wild-type and *cdc*25-mutant strains were incubated at 36°C for 4 hrs after logarithmic growth at 25°C.

Q.1.B.1. (3 points) By observing the phenotypes of the cultures W25, W36, M25 and M36, what can we conclude?

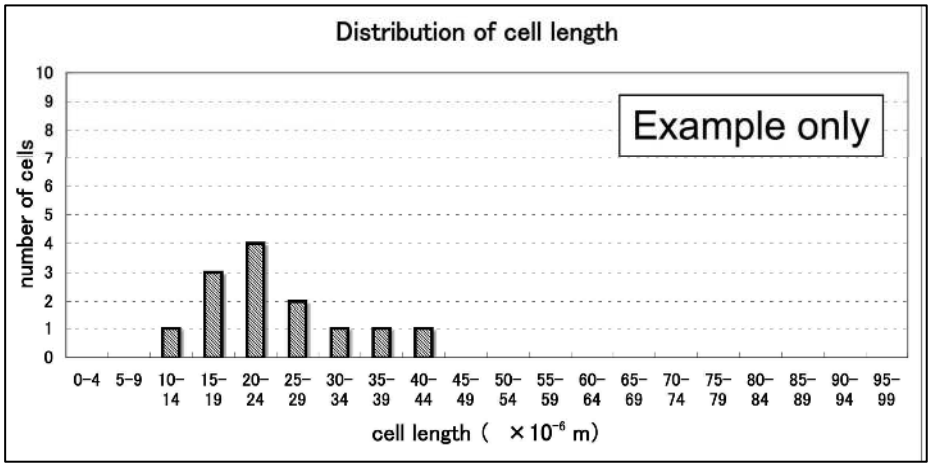
	Condition	Most of *cdc25* mutant cells	Wild type cells
A	25°C	Do not undergo cytokinesis	Undergo cytokinesis
B	25°C	Undergo cytokinesis	Do not undergo cytokinesis
C	36°C	Do not undergo cytokinesis	Undergo cytokinesis
D	36°C	Undergo cytokinesis	Do not undergo cytokinesis
E	25°C and 36°C	No significant difference in cytokinesis between *cdc25* mutant and wild type cells	

Q.1.B.2. (4 points) To measure cell length, your microscope is equipped with a micrometer in the eyepiece lens. In order to calibrate the eyepiece micrometer, a second micrometer, called the stage micrometer, is place on the stage of the microscope. The distance between any two adjacent lines on the stage micrometer is known to be 10.0 μm. By matching the lines on both micrometers, we can determine the distance between two adjacent lines of the eyepiece micrometer. Determine this distance in μm to two decimal places using the figure shown below.



Q.1.B.3. (12 points) Measure the longitudinal length of more than 10 cells selected at random in culture media of M36. Graph your results in the Answer Sheet according to the example indicated below. The scale of your eyepiece micrometer is 4 μm. Do not forget to

indicate the unit of length.



Q.1.B.4.(2 points) What can you conclude from your observations of each culture? *cdc25* cells are longer than wild-type cells at:

A both 25°C and 36°C.
B 36°C but not 25°C.
C 25°C but not 36°C.
D There is no significant difference in cell length between wild-type and *cdc25* cells at both 25°C and 36°C.

Part C

The following experiment was done using wild-type cells and 5 mutant strains (A-E). These mutant strains grow at 25°C as well as wild-type cells but are not able to grow at 36°C. All cells undergoing logarithmic growth at 25°C were then incubated at 36°C for an additional 4 hrs before chemical fixation. Fixed cells were stained with both Calcofluor (stains septa) and DAPI (stains DNA) for observation using fluorescence microscopy (as seen in the photograph provided on the bench).

Q.1.C.1.(10 points) The following statements describe the phenotype of the mutants incubated at 36°C. Identify the descriptions that correspond with each of the mutant strains (A-E), respectively.

1. Cytokinesis is repeated independently of progression of the cell cycle.
2. Cell cycle progresses but cytokinesis has not begun.
3. Cell cycle is arrested at interphase.
4. Karyokinesis is severely defective.
5. Completion of cytokinesis is suppressed.

Task 2. Study on the Motile Mechanism of Unicellular Algae (30 points)

Introduction

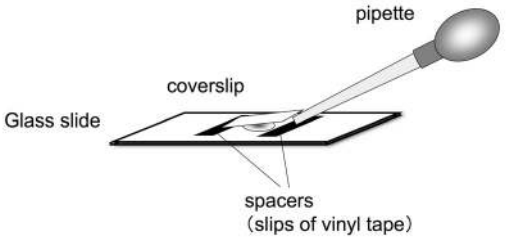
Some unicellular algae and zygotes of multicellular algae swim actively. This behavior is important for migration to appropriate conditions for growth and sexual reproduction. *Chlamydomonas reinhardtii*, an unicellular green alga, swims using flagella movement. Flagella often fall out when in contact with some stimuli, and some are absorbed into the cell body at a specific stage of the cell cycle.

This task concerns the machinery of flagella movement and flagella regeneration in *C. reinhardtii*.

Materials and equipment	**Quantity**
1. *C. reinhardtii* wild-type cells (wt)	1
2. *C. reinhardtii oda1*mutant (oda)	1
3. *C. reinhardtii pf17* mutant (pf)	1
4. Microscope	1
5. Box of glass slides	1
6. Box of glass coverslips	1
7. Acetic acid solution (A)	1
8. Neutralizing solution (N)	1
9. Disposable pipette（1 ml）	10
10. 1.5 ml microtube	5
11. Vinyl tape	1
12. Scissors	1

Caution

C. reinhardtii flagella frequently stick to glass slides. As a result, the swimming ability of the cell is hindered. Therefore, cells immobilized on a glass slide should be excluded from observations for cell movement. It is recommended to make a chamber as indicated below for the observation. Slips of vinyl tape are stuck on a glass slide in parallel, and a coverslip is mounted on the slips after the samples are loaded by pipette. This chamber will provide a space for the cells to swim.



Part A

Microscopically compare the wild-type (wt) and *pf17* mutant (pf) cells. This mutant has a normal shape and cellular structure but lacks a component of the radial spoke head in its flagella.

Q.2.A.1. (6 points) In comparison to wild-type cells, *pf17* mutant cells:

A swim in the same manner
B swim but more slowly
C swim but more rapidly
D do not swim at all

Q.2.A.2. (2 points) What can you conclude about the function of the radial spoke head?

A essential for flagella movement
B no effect on flagella movement
C suppresses flagella movement
D coordinates flagella movement

Part B

Microscopically compare the wild-type (wt) and *oda1* mutant (od). This mutant lacks a kind of dynein in flagella whereas the shape and other cellular structures are normal.

Q.2.B.1. (6 points) In comparison to wild-type cells, *oda1* mutant cells swim:

A in the same manner
B more slowly and smoothly
C more slowly and jerkily
D more rapidly and smoothly
E rapidly and jerkily

Q.2.B.2. (2 points) What can you conclude about the function of the dynein lost in the *oda1* mutant?

A essential for flagella movement
B no effect on flagella movement
C increases flagella movement
D coordinates flagella movement

Part C

Study the effect of acetic acid on flagella as follows:

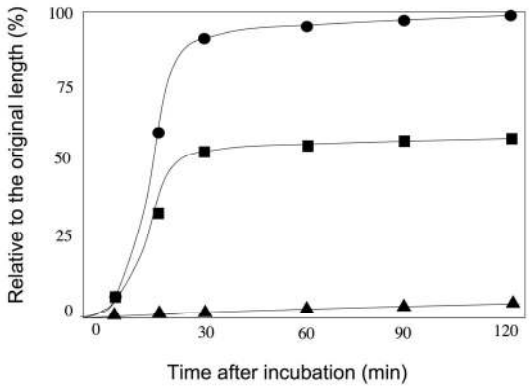
(i) Measure the percentage (A) of wild-type cells with flagella in 20 cells.
(ii) Transfer about 1 ml of the culture selected in (i) into a 1.5 ml microtube by disposable pipette, and add one drop of acetic acid solution
(iii) Add one drop of neutralizing solution after 30 seconds
(iv) Measure the percentage (B) of cells containing flagella in 20 cells after the treatment

Q.2.C.1.((4points x 2)=8 points) Calculate the percentage of cells containing flagella in the pretreatment (A) and posttreatment (B) samples.

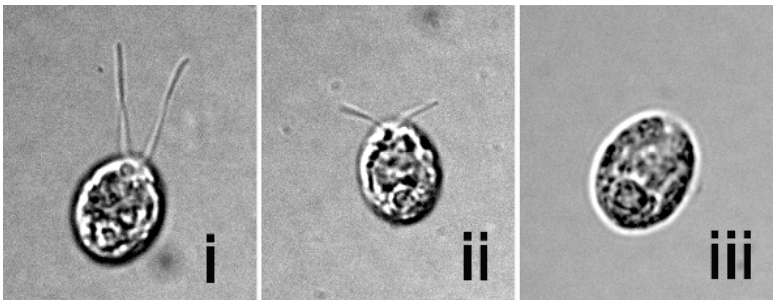
Part D

Wild-type cells with their flagella removed were incubated under different conditions (i, ii or iii). The following graph indicates the flagella length relative to its original length at different time points.

(i) control (incubated without inhibitors) (●)
(ii) incubated with cycloheximide, an inhibitor of protein synthesis (■)
(iii) incubated with colchicine, an inhibitor of microtubule formation (▲)



In addition, photographs of cells after incubation for 120 min are shown.



Q.2.D.1.(4 points) Are the following statements supported by observing the results of cells incubated with cycloheximide? Put a cross mark (x) in the appropriate boxes in the answer sheet.

1. All proteins incorporated in regenerated flagella are synthesized *de novo*
2. Regenerated flagella show no motility because of a lack of dynein
3. *De novo* synthesis of protein is essential for the complete regeneration of flagella.
4. *De novo* synthesis of protein is essential for the formation of the basal body of flagella

Q.2.D.2.(2 points) Based on your observations of cells incubated with colchicines, what is required for the regeneration of flagella?

A Polymerization of tubulin
B Polymerization of actin
C Polymerization of keratin
D Depolymerization of tubulin
E Depolymerization of actin
F Depolymerization of keratin

**********END OF PRACTICAL TEST 4**********