Country Code: _____ Country: _____

20th INTERNATIONAL BIOLOGY OLYMPIAD
12th – 19th July, 2009
Tsukuba, JAPAN

Directions :

- You should not open the envelope until the bell rings once to indicate the start of the test.
- After the bell rings, please open the envelope and write your student code on every page of the ANSWER SHEET at the beginning of the test.
- 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.
- When the bell rings twice to indicate the end of the test, please put down your pencil and stop writing.
- For safety reasons, do not take any food or drink into the laboratory.
- You must wear your coloured laboratory coat together with appropriate clothes and shoes.

・Please properly use the materials (pencils, a pencil sharpener, an eraser, a ruler, a marker pen, a stopwatch, goggles, gloves, a calculator) which were given to you at registration.

・Distilled water (DW) in a bottle, paper towels, cleaning papers and two plastic cups for discarding liquid and solid materials have been provided at your bench. Please use them as needed.

・After the test, please be sure that you have cleaned the bench before you leave.

How to handle a micropipette:

Each micropipette has a fixed range of volumes as indicated on the head of pipette. Please use appropriate types of the micropipettes. Do not cross the limits of this range.

Type (Volume)	Head	Window	Volume
P1000 (200-1000 microlitre)			indicates 850 microlitre
P200 (50-200 microlitre)			indicates 150 microlitre
P20 (2-20 microlitre)			indicates 15 microlitre
P2 (0.2-2 microlitre)			indicates 1 microlitre

Volume adjustment: turn the dial (1) to set the value to the desired volume, which can be seen in the window.

Use: Secure the pipette tip to the tip holder (2). Gently push down the plunger (3) to the first stop, hold, and dip the tip into the solution vertically to a depth of 2 - 4 mm. Release the plunger slowly and make it return to the original position. Remove the pipette from the liquid and transfer the contents to the desired tube. Make sure that the tip is close to the inner wall of the tube. Push the plunger to the first stop and then push further to discharge the solution completely from the tip. Remove the pipette from the tube. Put the used tip into the disposal container by pressing the tip-ejector (4).

Attention: With the 200-1000 microlitre pipette (P1000), it may a suck the solution into the pipette cylinder with the rapid release of the plunger. If this happens, please tell the help staff after the test.

Student Code: _____

20th INTERNATIONAL BIOLOGY OLYMPIAD

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PRACTICAL TEST 4

CELL PHYSIOLOGY

Total Points: 91

Duration: 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 (61 points)

Study on the cell cycle

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.

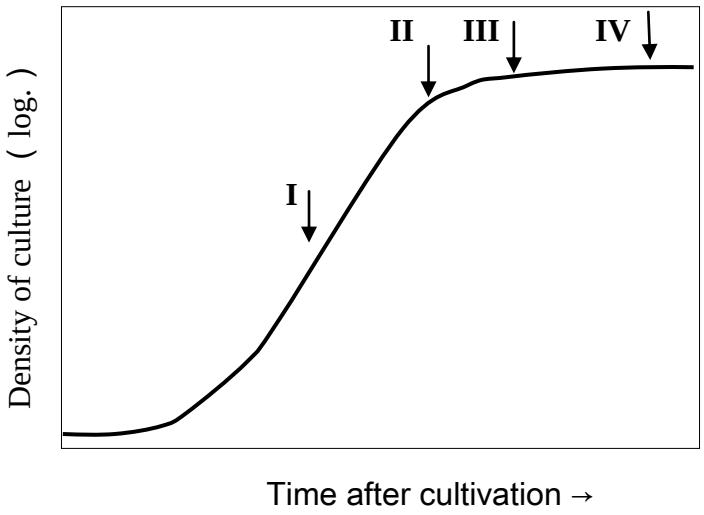
The following task involves examining cell proliferation using *S. pombe*.

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 μ L)	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.

Growth curve of *S. pombe*



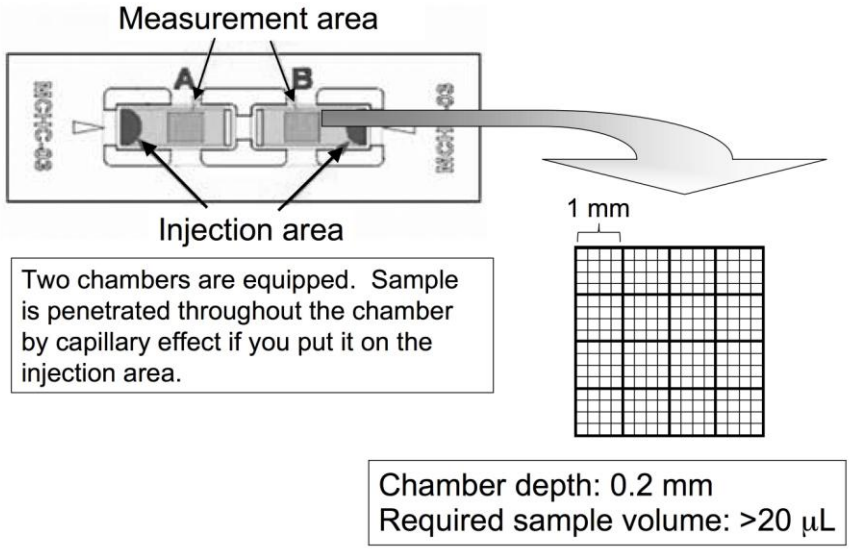
Q.1.A.1. (2x2points) Compare the cells in sample a with those in sample b, and answer the following questions.

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?

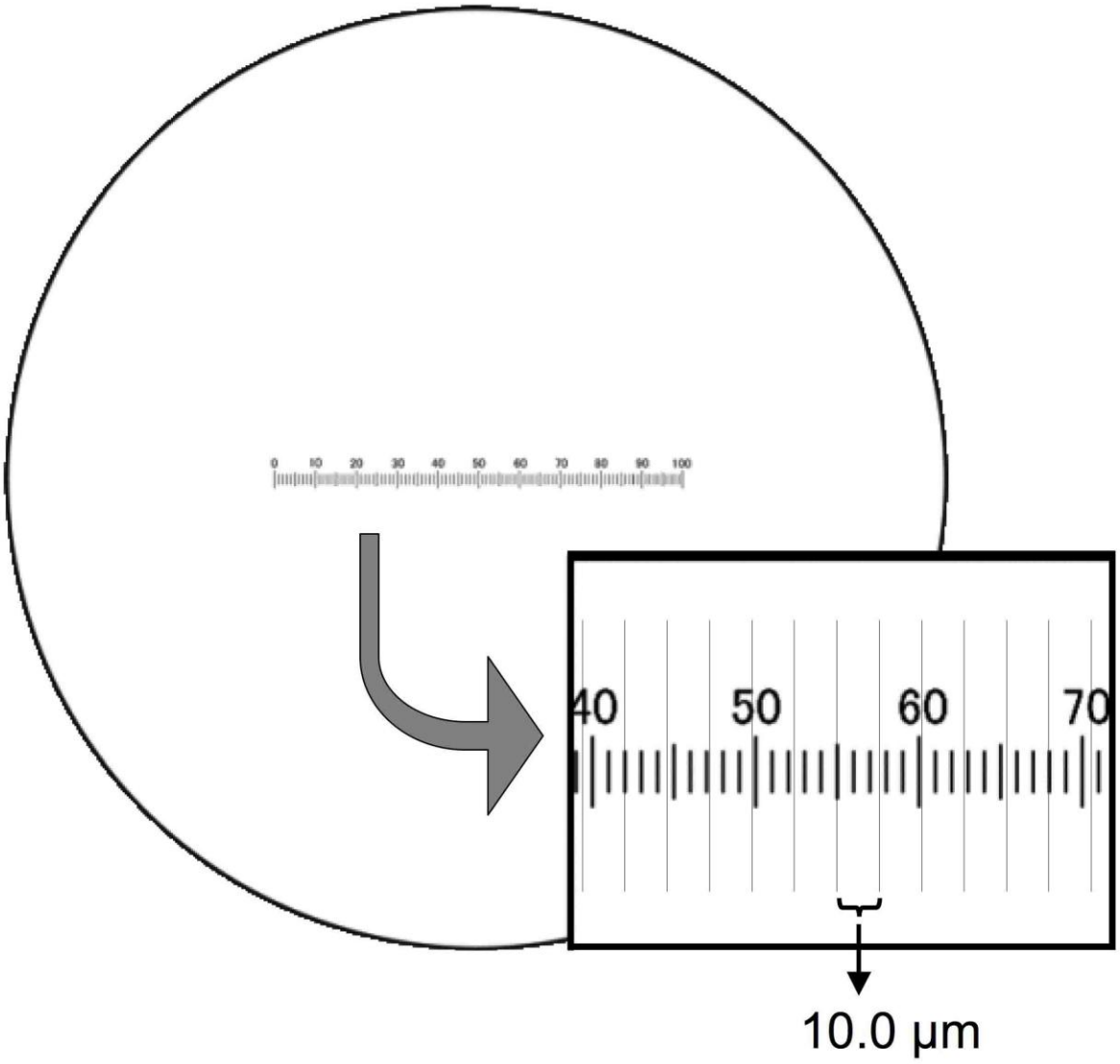
Part B

Both wild-type and *cdc25*-mutant strains were incubated at 36°C for 4 hrs after logarithmic growth at 25°C.

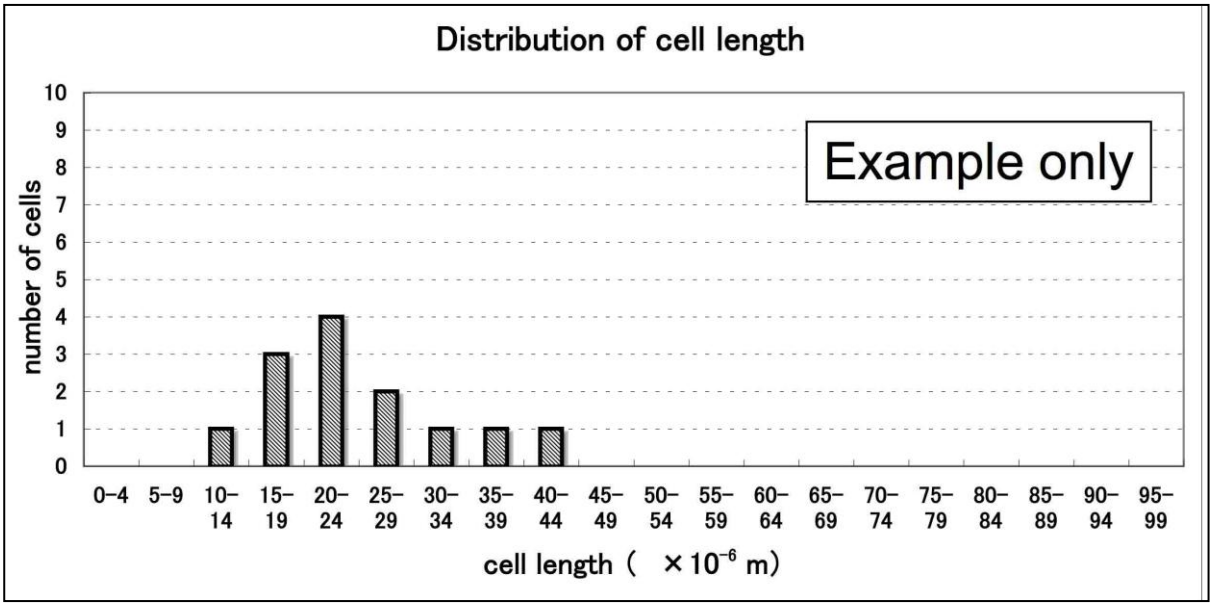
Q.1.B.1.(3 points) By observing the phenotypes of the cultures W25, W36, M25 and M36, what can we conclude?

	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 μ m. By matching the lines on both micrometers, we can determine the distance between two adjacent lines of the eyepiece micrometer. Determine this distance in μ m 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 μ m. 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 (30 points)

Study on the motile mechanism of unicellular algae

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.

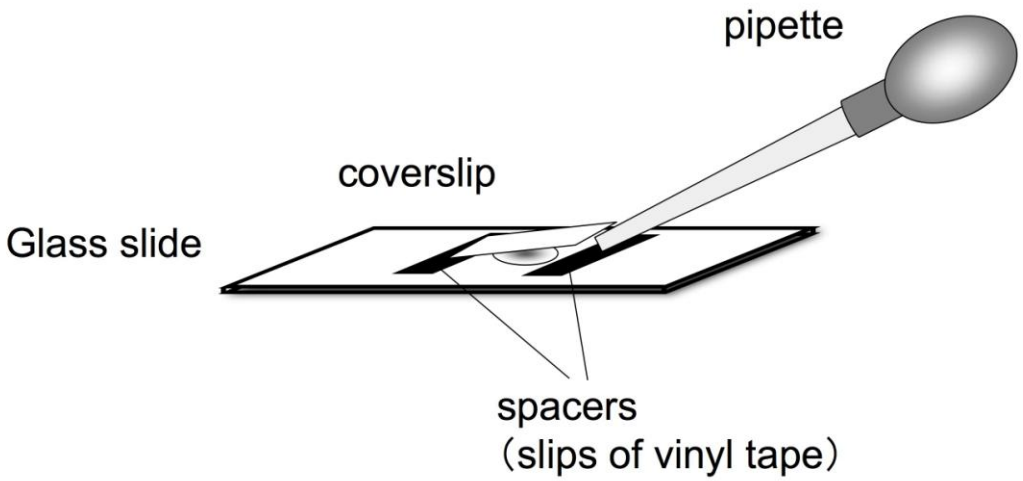
This task concerns the machinery of flagella movement and flagella regeneration in *C. reinhardtii*.

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.

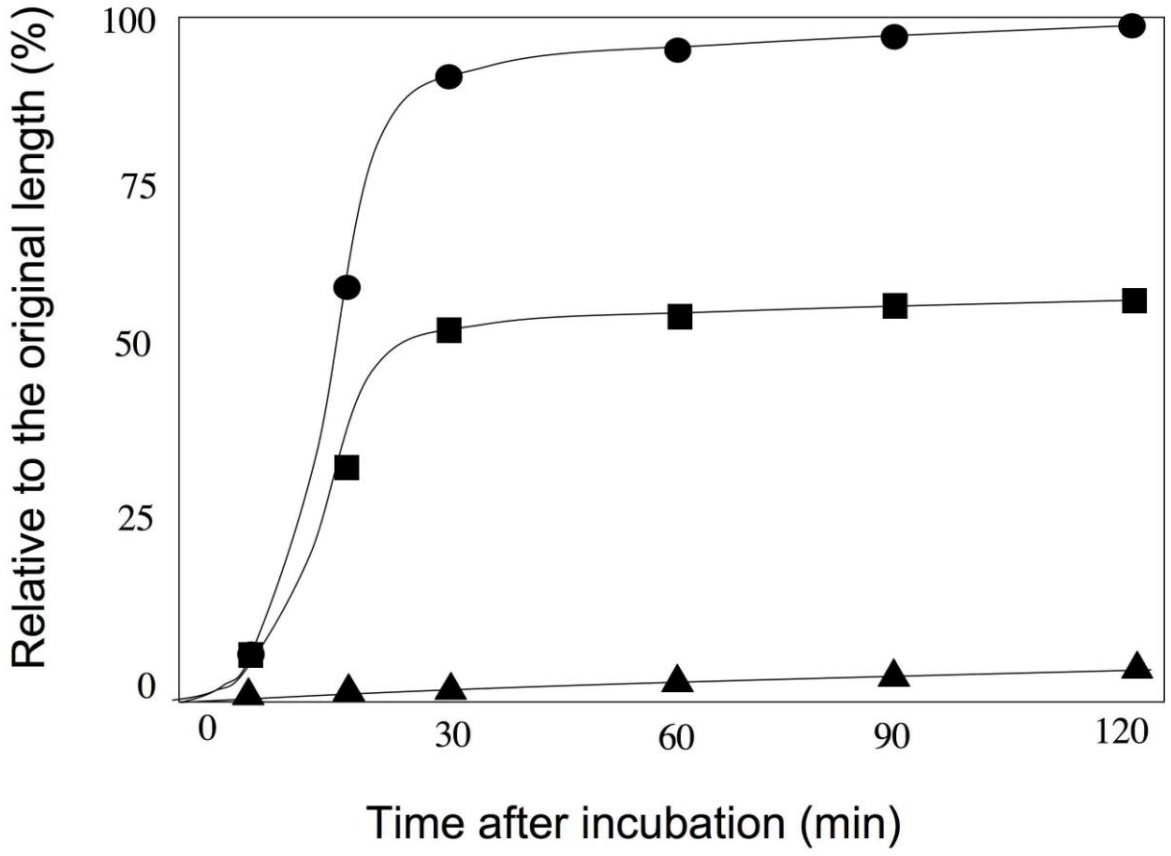
Part D

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.

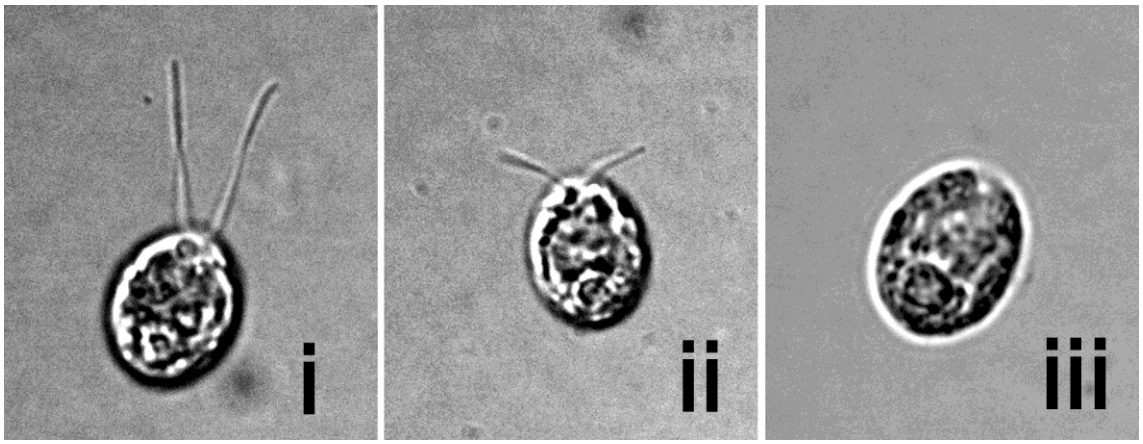
(i) control (incubated without inhibitors)（●）

(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

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CELL PHYSIOLOGY

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Duration: 90 minutes

ANSWER SHEET

STUDENT CODE:

Q.1.A.1. (2x2 points)

1	
2	

Q.1.A.2. (6 points)

cells/ml

Q.1.A.3. (5 points)

Total cells counted	%

Q.1.A.4. (4 points)

formula	solution
	min

Q.1.A.5. (3 points)

Q.1.A.6. (8 points)

a	b	c	d

STUDENT CODE:

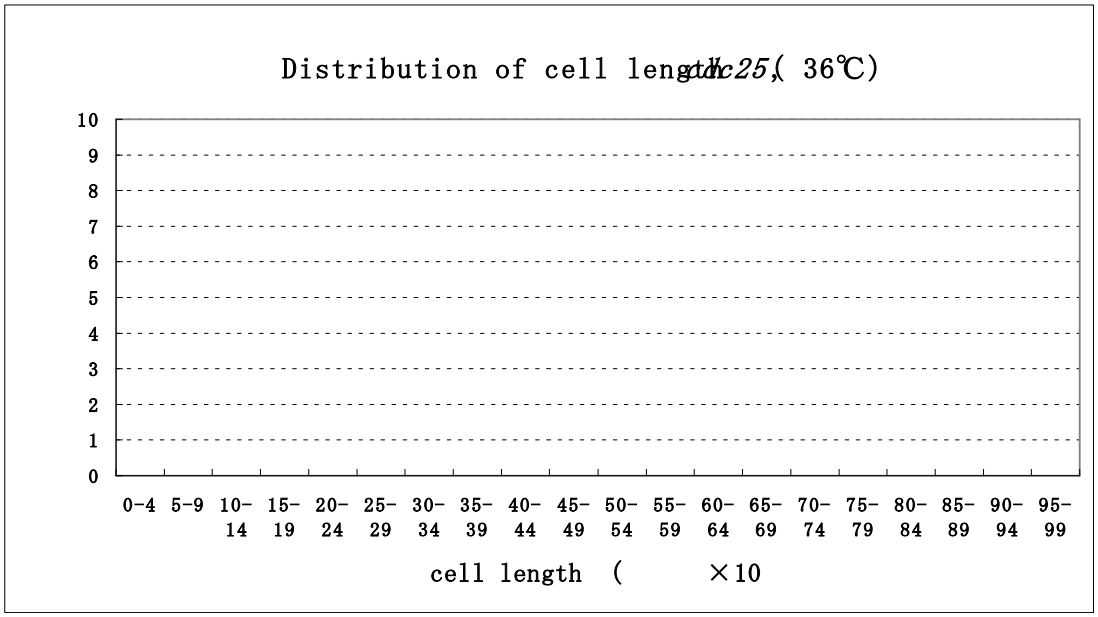
STUDENT CODE:

Q.1.B.1.(3 points)

Q.1.B.2.(4 points)

μm

Q.1.B.3.(12 points)



Q.1.B.4.(2 points)

STUDENT CODE:

Q.1.C.1. (10 points)

1	2	3	4	5

STUDENT CODE:

Q.2.A.1. (6 points)

Q.2.A.2. (2 points)

Q.2.B.1. (6 points)

Q.2.B.2. (2 points)

Q.2.C.1. (4 points x 2=8 points)

A	B
%	%

Q.2.D.1. (4 points)

	Supported	Non-supported
1		
2		
3		
4		

Q.2.D.2. (2 points)

STUDENT CODE:

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