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: _____

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PRACTICAL TEST 3

GENETICS

Total Points: 98

Duration: 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 (9 points)

Phenotypic observation of mutant flies

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 (33 points)

Inheritance of white eye mutation

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 (18 points)

Separation of eye pigments

Materials and Equipment Quantity

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

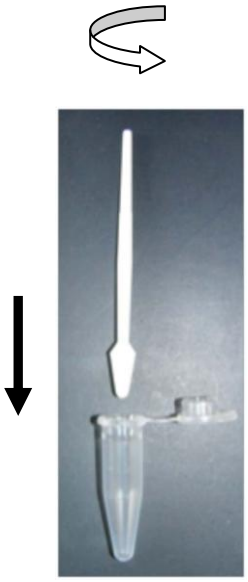
1. 1.5 ml tubes (8) and (9) containing eye-pigments extraction solution	1 set (1 spare set)
2. Empty 1.5 ml tubes (10) and (11)	1 set (1 spare set)
3. Micropestles (in 15 ml tube)	2 (1 spare)
4. Centrifuge	1
5. Micropipette (P20)	1
6. Pipette tips (for P200 and P20)	1 pack
7. Empty 1.5 ml tubes (no numbers 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 (14 points)

Reading chromatography

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.

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.

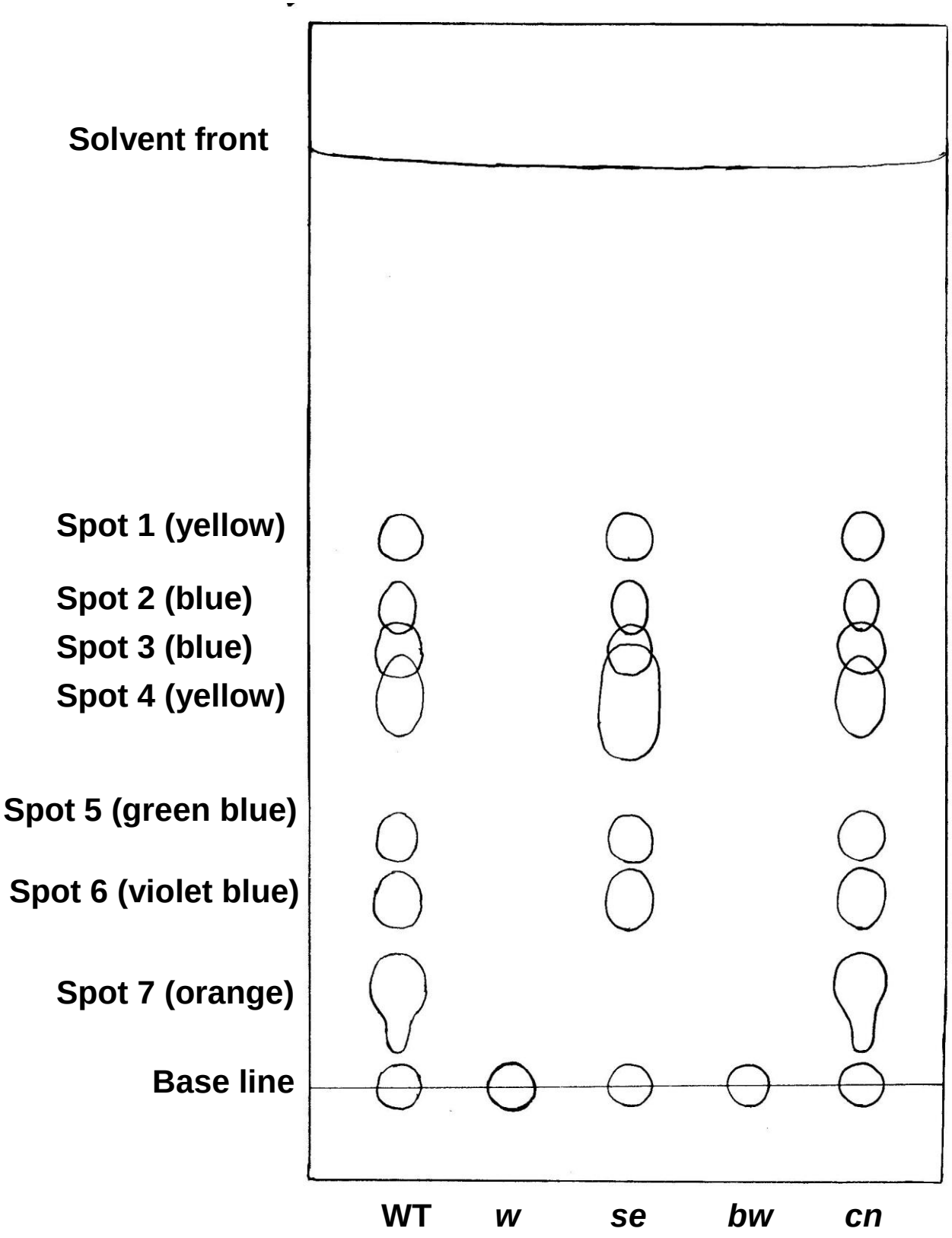


Figure 1. Chromatography of eye pigments from wild type and mutant flies

Task 5 (24 points)

Analysis of White Protein

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

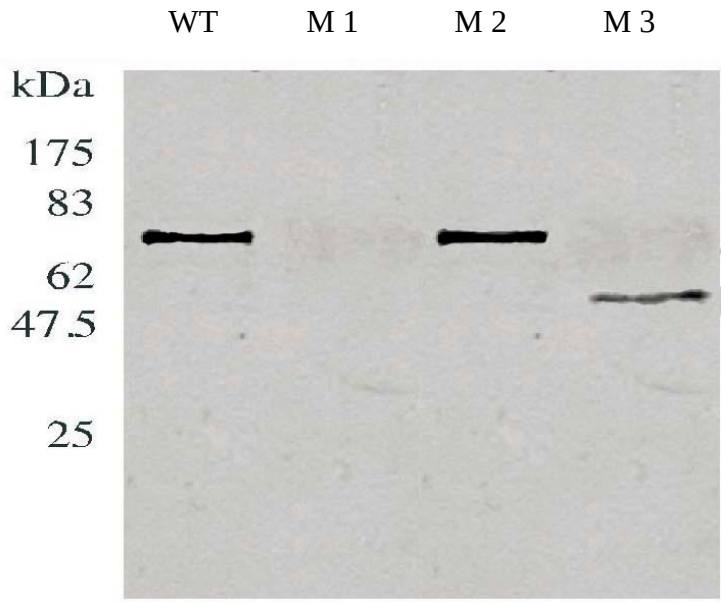
1. Add 50 µl protein extraction buffer (tube A) in the tube (10) (bodies of red-eyed flies) and (11) (bodies of white-eyed flies) prepared in Task 3. Crush the flies with the micropestle. Use different micropestles for wild type and mutant samples.
2. Centrifuge tubes (10) and (11) at 14,000 rpm for 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

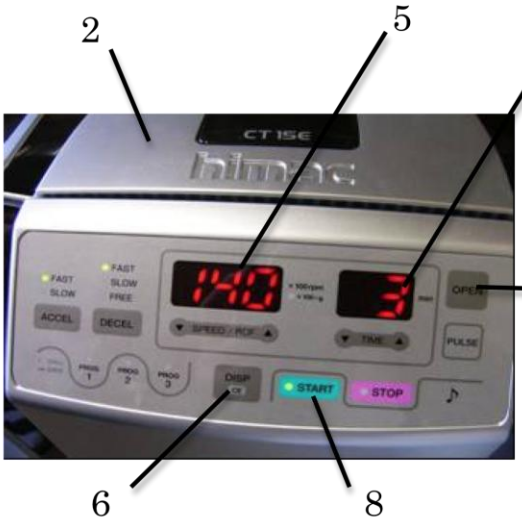


Figure 2

Figure 3

STUDENT CODE:

Student Code: _____

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12th – 19th July, 2009

Tsukuba, JAPAN

PRACTICAL TEST 3

GENETICS

Total Points: 98

Duration: 90 minutes

ANSWER SHEET

STUDENT CODE:

Q.1.1. (9 points)

(2)	
(3)	
(4)	

Q.2.1.(8 points)

	red females	white females	red males	white males
(5a)				
(5b)				

Q.2.2.(8 points)

	red females	white females	red males	white males
(6a)				
(6b)				

Q.2.3.(8 points)

	red females	white females	red males	white males
(7)				

STUDENT CODE:

Q.2.6. (9 points)

(5a) and (5b)	
(6a) and (6b)	
(7)	

Task 3 (18 points)

Q.4.1. (5 points)

Spot No.	Pigment (A-G)	WT	*w*	*se*	*bw*	*cn*
1		+				
2		+				
3		+				
4		+				
5		+				
6		+				
7		+				

Q.4.2. (9 points)

se	

STUDENT CODE:

bw	
cn	

Photograph of the gel (18 points)

Q.5.1. (3 points)

M1	M2	M3

Q.5.2. (3 points)

M1	M2	M3

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