Country Code: _____	Student Code: _____

The 21st INTERNATIONAL BIOLOGY OLYMPIAD

11th – 18th July, 2010

Changwon, KOREA

PRACTICAL TEST 3

GENETICS AND CELL BIOLOGY

Total Points: 50

Duration: 90 minutes

Dear Participants,

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

  Task I (35 points)

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

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

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

- Write down your results and answers in the **Answer Sheet. Answers written in the Question Paper will not be evaluated.**

- Please make sure that you have received all the materials listed for each task. If any of the listed items is missing, please raise your hand.

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

Good Luck!!

GENETICS AND CELL BIOLOGY

This practical test is composed of 2 tasks.

TASK I. (35 points)

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

(2) Characterization of the relationship between genotypes and phenotypes

This task is composed of 2 parts.

Materials and Equipments

On individual Table

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

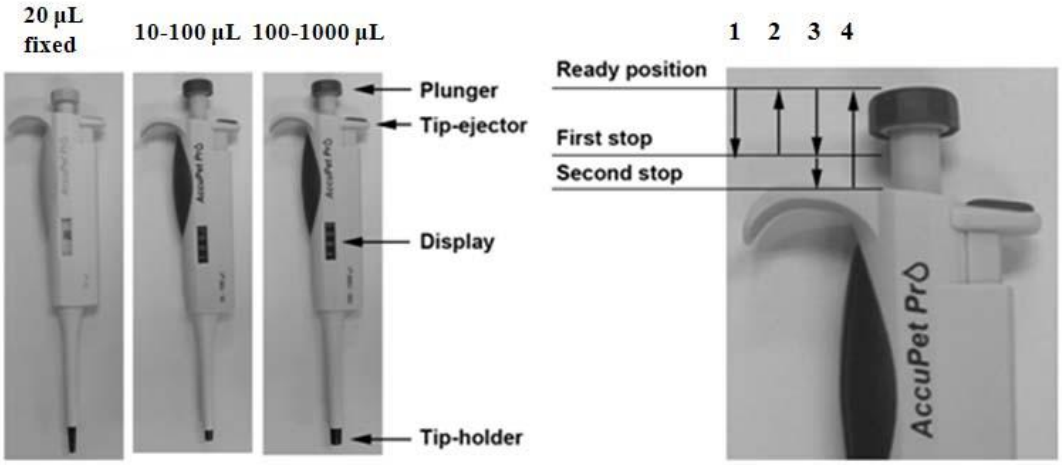
Label	Treatment	Label	Treatment
WT-0	Plant WT + distilled water		
WT-1	Plant WT + 1 μM hormone H	WT-100	Plant WT + 100 μM hormone H
dA-1	Plant dA + 1 μM hormone H	dA-100	Plant dA + 100 μM hormone H
dAB-1	Plant dAB + 1 μM hormone H	dAB-100	Plant dAB + 100 μM hormone H
dABC-1	Plant dABC + 1 μM hormone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

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

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

1. Gel documentation system equipped with a UV source

Handling of Micropipettes



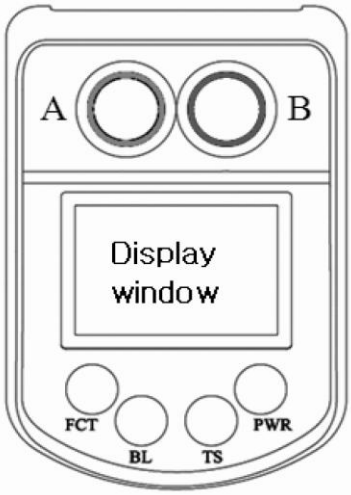
Adjustment method

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

Usage method

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

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



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

Usage method

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

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

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

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

Operating Instruction for the DNA Gel Electrophoretic Apparatus

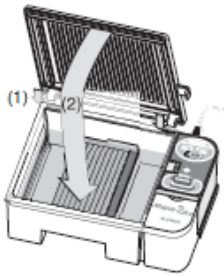
1) Load the samples to the wells using the 20 μL micropipette.

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

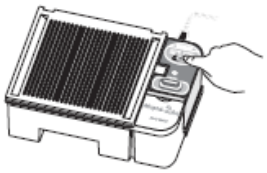
Do this as follows:

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

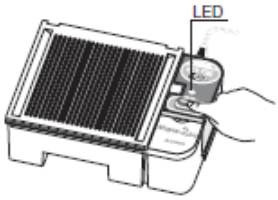
(2) Then, rotate the cover forward to close it.



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



4) Push the operation switch to start the migration.

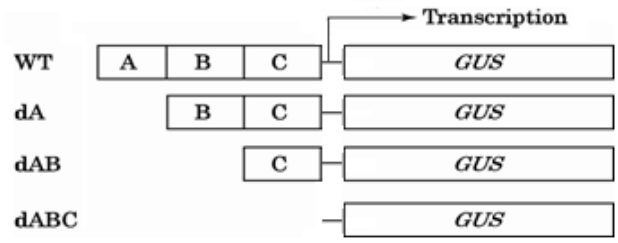


5) In this experiment, the gel running time should be 30 min. Make sure to turn the operation switch OFF when the running is finished.

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

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

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



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

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

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

Measurement of fluorescence and determination of MU amount

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

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

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

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

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

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

$Y = 0.04 X + 2.5$

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

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

Measurement of absorbance at 595 nm and determination of protein amount

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

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

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

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

$Y = 98X + 2.8$

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

Calculation of GUS activity

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

Table 1 is worth of 9 points.

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

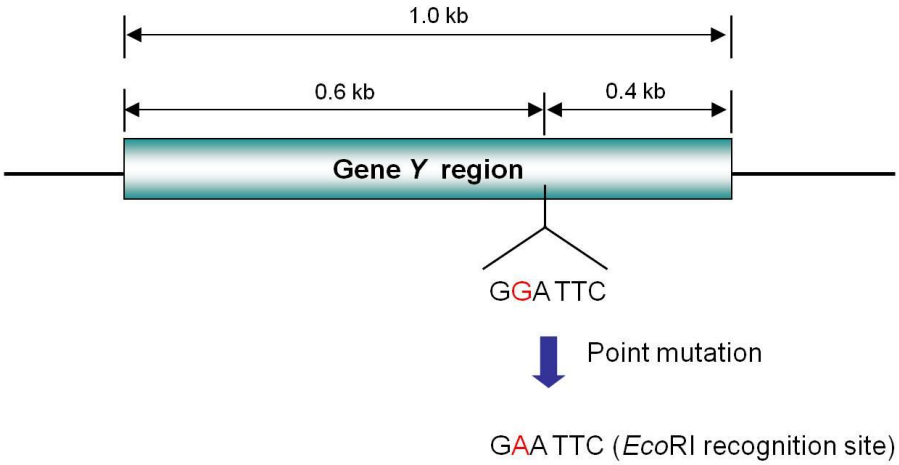
Note: - stimulation: more than **3-fold** increase in gene *X* expression
- no effect: less than **3-fold** increase in gene *X* expression

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

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

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

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



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

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

Genotyping of gene *Y* by gel electrophoresis

Note: Always wear polygloves during the experiment !!!

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Background

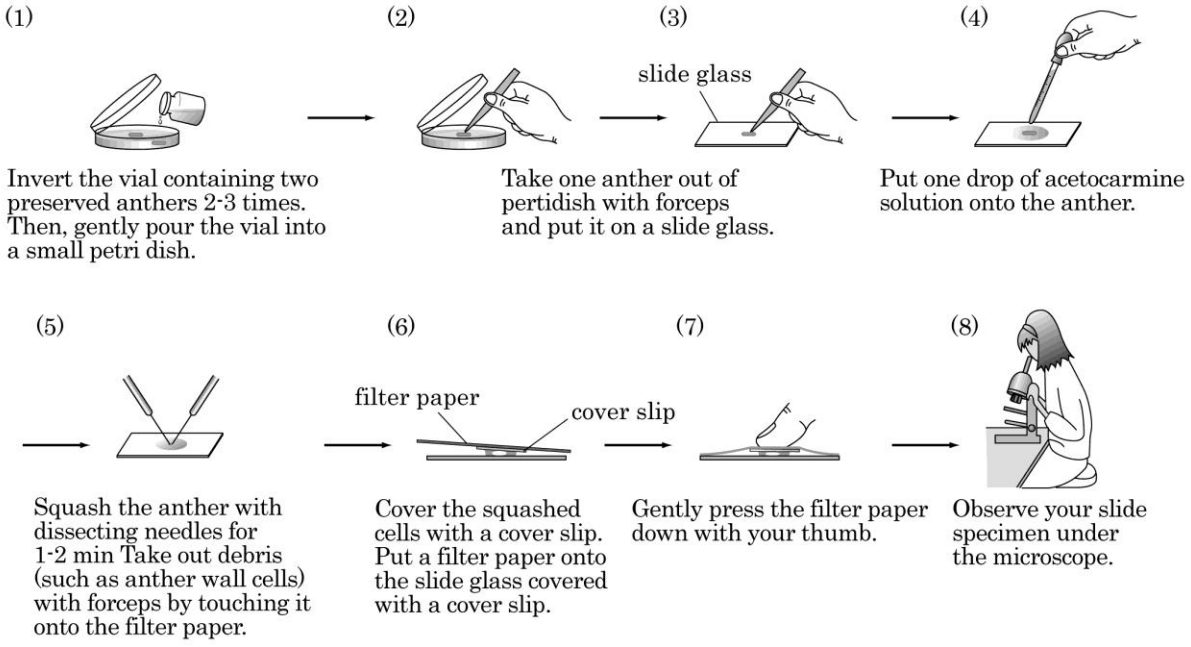
Using a light microscope, you will observe meiotic cells in preserved rye anthers. Anthers at a specific stage of meiosis were selected and were preserved in 70% ethanol.

Requirements – Overview

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

Procedure

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



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

Notes :

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

Q3. Answer the following questions.

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

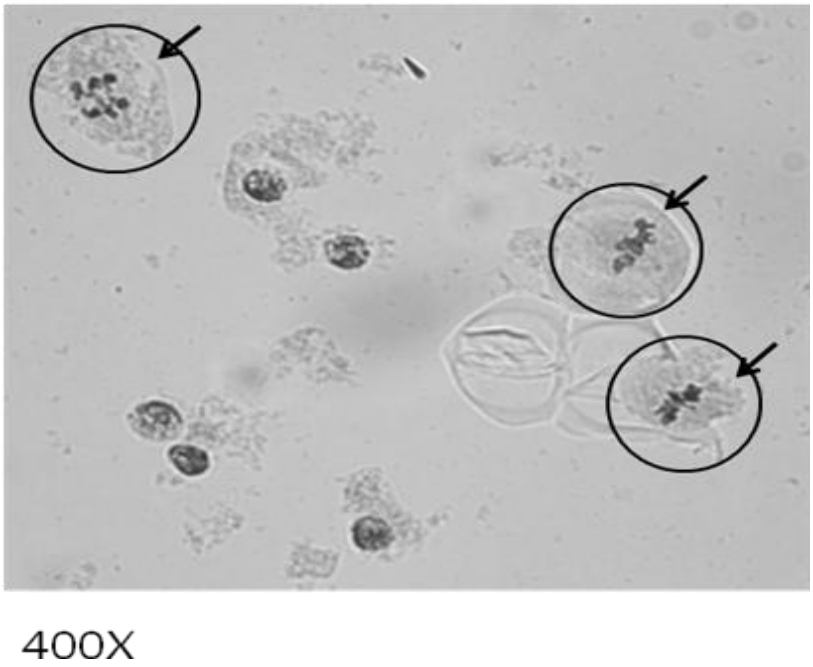


Figure Q3. Examples of cells undergoing meiotic cell division observed under a microscope.

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

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

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

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

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

PRACTICAL TEST 3

Answer Key

GENETICS AND CELL BIOLOGY

Total Points: 50

Duration: 90 minutes

TASK I. (35 points)

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Part I. (20 points)

Table 1. (9 points=1X9)

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

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

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

1. Values of measurements and calculations were verified to be within ranges indicated in the Table 1, the Answer Key.
2. Any mistake (s) in measurements and calculations for each plant will be considered as a wrong answer.

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

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

1. Any correct answers that are not supported by the measurement and calculation data in Table 1 will be considered as wrong answers.
2. Plural choices for each hormone-treated plant are null.

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

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

Plural choices for each promoter region in null

Q1.3. (1 point)

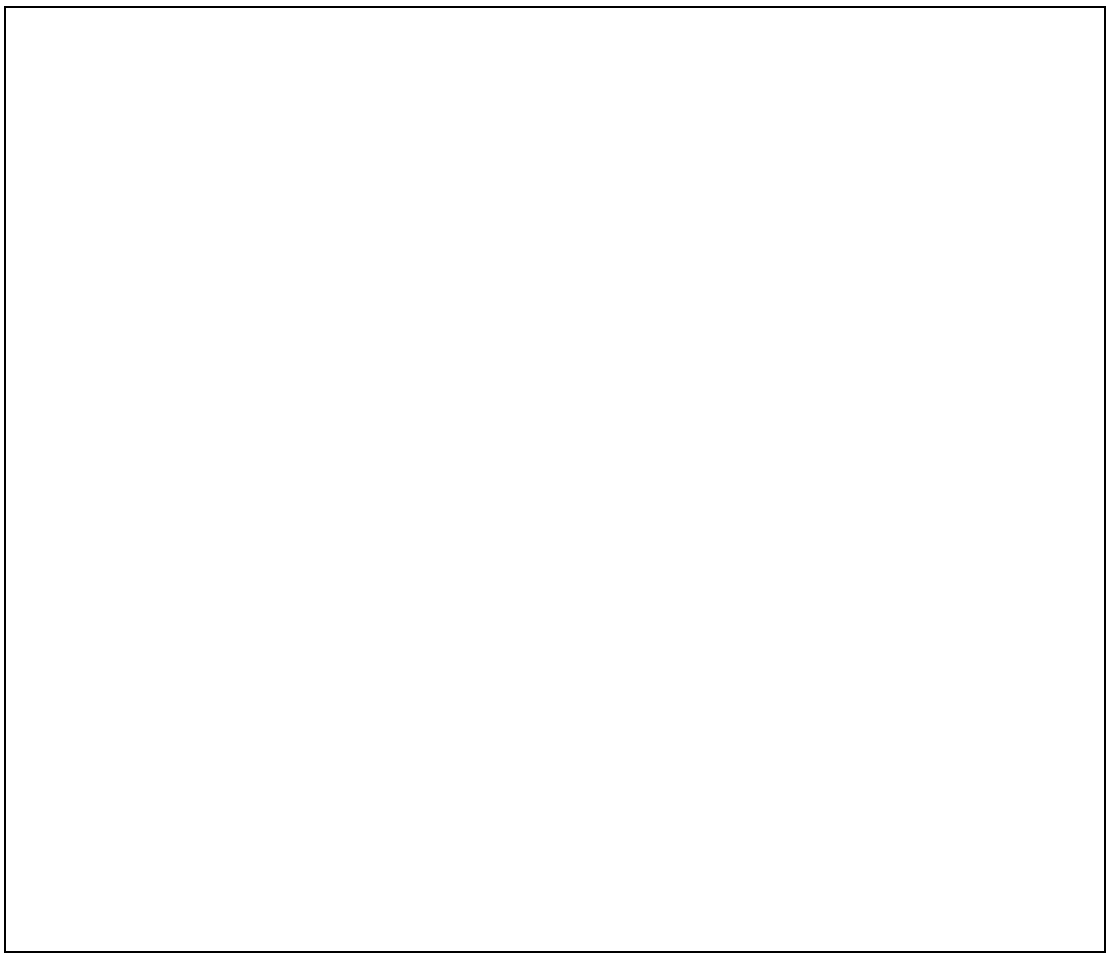
Action mode	
Transcriptional positive feedback regulation	
Transcriptional negative feedback regulation	√

The correct answer that is not supported by the answers for WT-1 and WT-100 in **Q1.1** (stimulation and no effect, respectively) will be considered as a wrong answer.

Part II. (15 points)

Q2.1. (3 points)

<Attach the agarose gel picture here>



1. Expected electrophoresis results even though a part of results is not perfect: 3 points
2. Good electrophoresis results about the ten DNA samples but some mistakes: e.g. wrong loading order, empty well(s): 2 points

3. Missed one sample

    (1) Marker missed: 2 points

    (2) Plant sample DNA missed: 1 point

4. Missed more than one sample: 0 point

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

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

Q2.3. (2 points)

Characteristic of the mutation	Dominant	√
	Recessive	

Q2.4. (2 points)

Probability of dwarf offspring	50 (%)

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

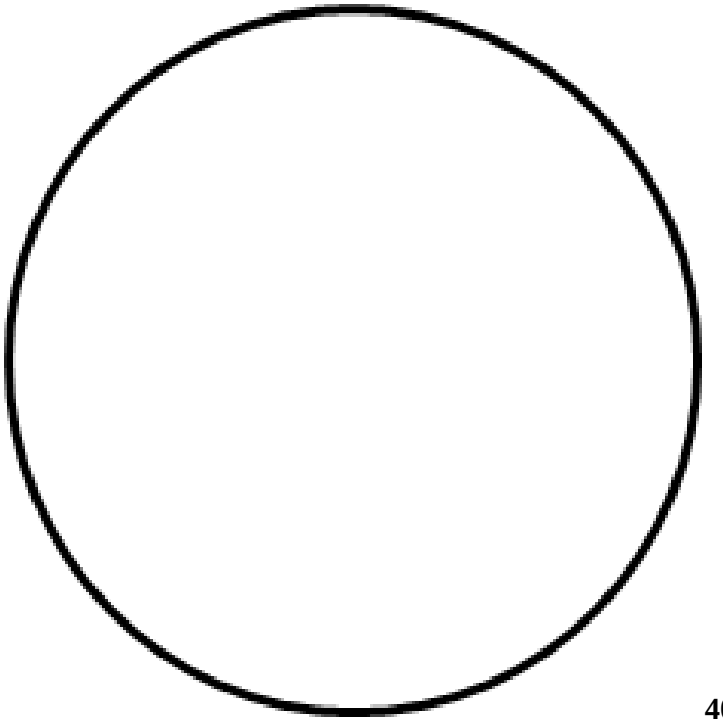
Number of heterozygous (*Yy*) offspring	3750
Number of dwarf offspring	4375

TASK II. (15 points)

Q3.1. (1 point)

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

Q3.2. (8 points)



400X

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1) 0 point will be given if you do not i) draw any cells, ii) draw just cells without any chromosomes, and iii) draw just cell debris.

2) 2 points will be given if you draw only anther wall cell(s).

3) Full points will be given if you draw a cell undergoing meiosis with proper chromosomes shapes.

Q3.3. (4 points)

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

Q3.4. (2 points = 1 × 2)

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

C: the amount of DNA in a haploid complement