Country Code: _____	Student Code: _____

The 21st INTERNATIONAL BIOLOGY OLYMPIAD

11th – 18th July, 2010

Changwon, KOREA

PRACTICAL TEST 3

實驗題 3

GENETICS AND CELL BIOLOGY

遺傳學與細胞學

Total Points: 50

總分：**50** 分

Duration: 90 minutes

時間：90 分鐘

Dear Participants, 敬愛的參賽者

- In this test, you have been given the following 2 tasks: 本試卷包含兩大題

  Task I (35 points)

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

  啟動子與基因表現之調控研究

  (2) Characterization of the relationship between genotypes and phenotypes (15 points)**.** 描述並比較基因型與表現型的關係

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

  觀察固定過的裸麥花粉囊中減數分裂細胞

- Write down your results and answers in the **Answer Sheet. Answers written in the Question Paper will not be evaluated.**在答案紙上作答，試題上的答案將不予計分。

- 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. 確認所有材料的種類與數量都與試題相同無誤，如有短缺，立刻舉手。

- Stop answering and put down your pencil **immediately** after the end bell rings. The supervisor will collect the Question Paper and the Answer Sheet. 鈴聲響時，立刻停止作答。監考老師會收取試題與答案紙。

Good Luck!! 助你好運

GENETICS AND CELL BIOLOGY

遺傳與細胞生物學

This practical test is composed of 2 tasks.

本實驗考試共計兩大題

TASK I. (35 points)

第一大題 (35 分)

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

啟動子與基因表現之調控研究

(2) Characterization of the relationship between genotypes and phenotypes

描述並比較基因型與表現型的關係

This task is composed of 2 parts.

本試題包括兩部分

Materials and Equipments

材料與儀器

On individual Table

個人設備列表

1. Fluoro-spectrophotometer

   螢光分光光度計

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.

   9 支 微量離心管，內含 50 uL 分別已經標識不同植物來源之萃取液。每一種各有兩支。(2 x 9 = 18 支)。透明的微量離心管適用於蛋白質濃度測定，深色的微量離心管適用於螢光測定。

Label 標籤	Treatment 處理	Label 標籤	Treatment 處理
WT-0	Plant WT + distilled water		
WT-1	Plant WT + 1 μM hormone H 植物 (激素 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)

   一支 15 mL 塑膠管內裝 12 mL 的 Bardford 溶液 (Bardford 溶液可以用來測定蛋白質濃度)

4. 1 mL of 1 mM MUG (fluorescence substrate to measure GUS activity) in a microfuge tube

   一支微量離心管內裝入 1 mL 的 1 mM MUG 溶液 (MUG 是一種螢光受質，可以用來測量 GUS 的活性)

5. 12 mL of stop reagent for the GUS (enzyme β-glucronidase which converts MUG into MU) reaction in a 15 mL plastic tube

   一支 15 mL 塑膠管內裝 12 mL 的 GUS 終止液 (GUS 是一種 β–glucronidase 酵素，可將 MUG 轉化成 MU)

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)

   2 支 DNA 片段大小標記管 (標記 M，各有 50μL)，8 支 含有 *Eco*RI 處理過的 DNA 片段 (標記為 P1~P8，各有 50μL)

7. Two microfuge tubes labeled as GUS BL and Pro BL, respectively.

   有 2 支微量離心管分別標記為 GUS BL 與 Pro BL。

8. Three micropipettes (one each for 10-100 μL and 100-1000 μL, and a fixed volume pipette for 20 μL)

   三支微量吸管 (兩隻可調整刻度，分別為 10-100 μL 與 100-1000 μL。一支為固定刻度，20 μL)

9. A box of yellow tips for the 20 μL and the 10-100 μL micropipettes

   黃 色微量吸管頭 1 盒 (可供 10-100 μL 與固定刻度 20 μL 微量吸管使用)

10. A box of blue tips for the 100-1000 μL micropipette

    藍 色微量吸管頭 1 盒 (可供 100-1000 μL 微量吸管使用)

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.

    DNA 電泳設備。內含 1% 瓊脂膠體，與 1X TAE 電泳緩衝液。**如果膠體有破裂不全，舉手告訴你的助教。**

12. A tip disposal container 微量吸管放置架。

13. Polygloves 橡膠手套。

14. 25 cuvettes for the Fluoro-spectrophotometer

    25 支比色管用於 螢光分光光度計用。

15. A calculator 計算機

16. A timer 計時器

17. A Scotch tape 膠帶

18. An ice bucket filled with ice 裝有冰塊的冰桶

19. Microfuge tube racks 微量離心管管架

20. Green card 綠色卡片

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

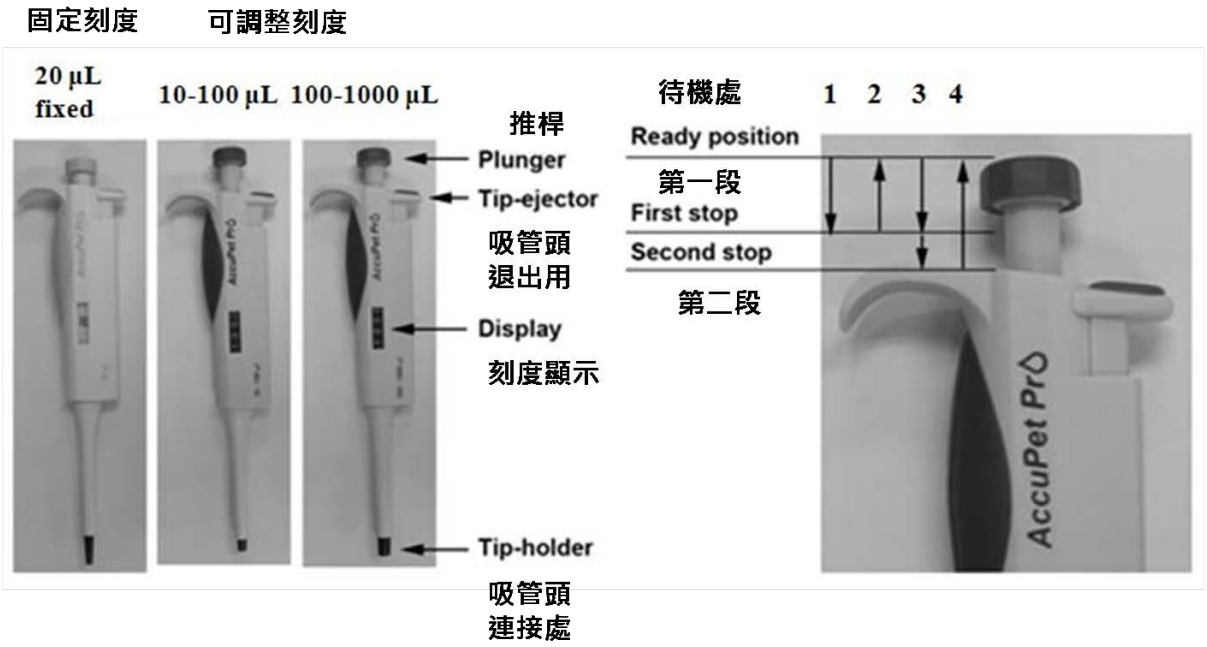
<u>共同儀器列表</u>

1. Gel documentation system equipped with a UV source

   含有 UV 光的膠體分析設備

Handling of Micropipettes

微量吸管使用法



Adjustment method 調整模式

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.

利用推桿順時針或逆時針旋轉調整所需要的體積。可於刻度顯示處看到所需刻度。每支微量吸管適用的體積被標記在刻度處顯示下方，請注意不要超過適用範圍。

Usage method 使用方法

1) Secure the pipette tip to the tip holder. Gently push down the plunger to the first stop.

   確實將吸管頭與微量吸管連接緊密，將推桿壓到第一段處。

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.

   將吸管頭移到液面下約 2~4 mm 處。輕輕放開推桿，讓他回到待機處。

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.

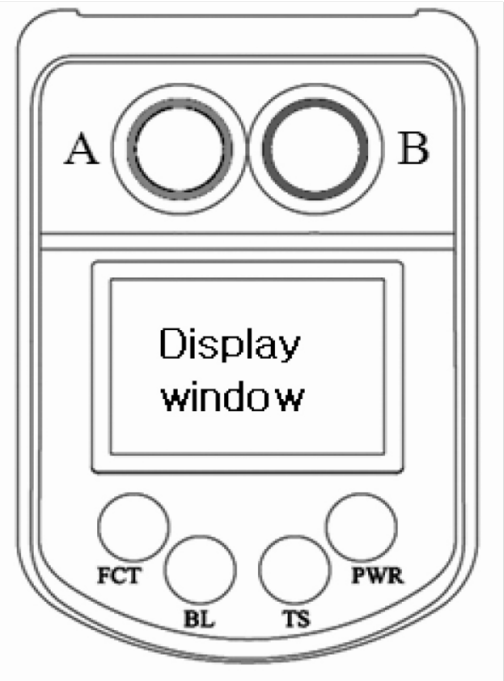
   將吸管頭移到所要添加的試管中，先將推桿壓到第一段，再用力壓到第二段處，直到液體完全離開吸管頭為止。

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.

   將吸管頭移開，利用吸管退出用按鈕將吸管頭退出。

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

螢光分光光度計操作說明 (測量 MU 螢光與 蛋白質測量 [595 nm 吸光值])



A: Cuvette holder for protein measurement
蛋白質測量處

B: Cuvette holder for fluorescence of MU measurement
MU 螢光測量處

FCT: Function key
功能鍵

BL: Blank key
空白鍵

TS: Test sample key
測量鍵

PWR: Power key
開關

Usage method 使用方法

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

重要事項：不要觸碰到比色管的光路徑。

1) Press the PWR ( ) button to turn on the machine. The display window will be turned on after a beep.

壓下電源開關【PWR ( )】，啟動機器。顯示螢幕會在 嗶 聲後顯示。

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).

為了要進行歸零，要將 空白 的比色管插入正確的位置 (蛋白質濃度在左邊 A 處，GUS 活性測量在右邊 B 處)。使用比色管的狀態會顯示在螢幕上 (為 A， 為 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.

注意：歸零所使用的樣本分別放在不同的微量吸管中。GUS BL 為 GUS 活性測定歸零用， ProBL 為蛋白質濃度歸零用。

3) Press the BL button, and the blank indicator ( ) will appear when the blank is set at 0.0.

壓下 空白鍵 (BL)，此時螢幕會顯示 符號，耐心等待，直到數字出現為 0.0 為止。

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 ()

為了要測量樣本，移出 空白 的比色管，插入待測的 樣本 比色管。(蛋白質濃度在左邊 A 處，GUS 活性測量在右邊 B 處)。壓下 測量鍵 (TS)。此時螢幕會顯示  符號，耐心等待，直到數字出現為止。

5) To end the machine, keep the PWR button pressed till beep is heard.

欲關機時，持續按下開關鍵 (PWR)，直到 嗶 聲出現即可。

Operating Instruction for the DNA Gel Electrophoretic Apparatus

DNA 膠體電泳操作說明

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

   使用 固定刻度 微量吸管 (20μL) 添加樣本。

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

   確認開關 (⏻) 是在 OFF，(如下圖) 後。關上蓋子。

Do this as follows:

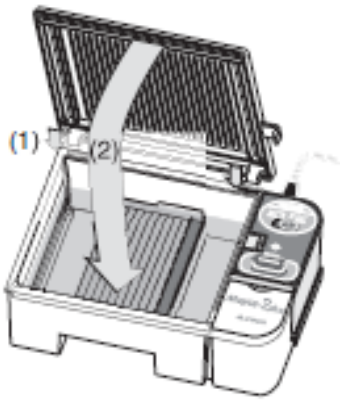
依下列步驟操作

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

首先，如下圖方向放置電泳槽，將上方的兩個卡榫對準插入。

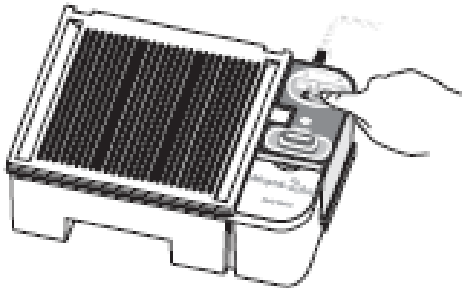
(2) Then, rotate the cover forward to close it.

如下圖，放下蓋子。



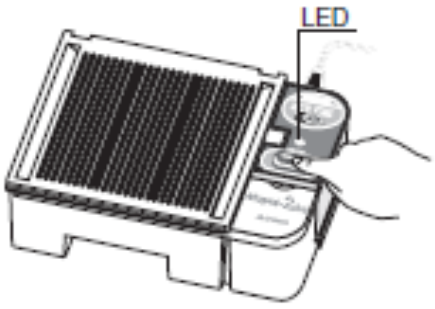
3) Set the voltage to “Half” using the output selection switch.

如下圖，將伏特 (VOLTAGE) 調整到 HALF (左邊)



4) Push the operation switch to start the migration.

5) 如下圖，按下開關，開始電泳。



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

本實驗中，當電泳時間 達到 30 分鐘時，即可將電源關閉。

Part I. (20 points) 第一部分 (20 分)

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.

使用已 融合 **GUS** 報導基因之 **X** 基因進行 激素對基因表現的影響，並分析啟動子中激素反應單元的特性

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 a hormone-responsive transcription factor(s) that binds specifically to this region, resulting either in gene activation or suppression.

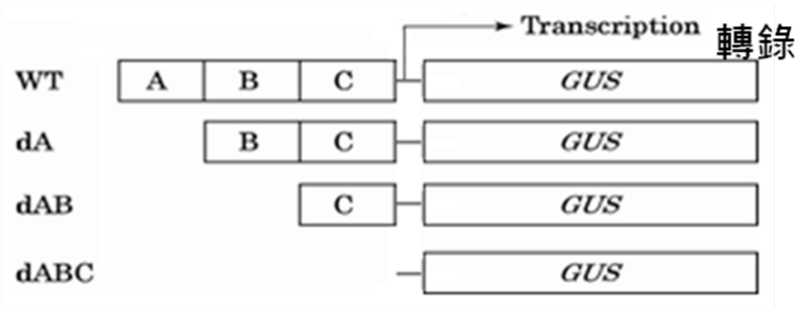
植物對於激素的反應是依靠 激素反應基因 進行。在基因的啟動子位置，一段被稱為 ***cis* -** 單元 的 DNA 序列會反應基因表現的調控時間與表現量。基因調控受制於該區域是否受到 激素反應 轉錄因子 的活化或抑制。

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).

本試題單元，將檢視阿拉伯芥中的激素，透過 基因反應途徑 對 *X* 基因的調控。為找出啟動子中激素反應之區域以了解 X 基因受激素調控之模式，吾人把 *X* 基因 的啟動子位置分別區分成 A~C 三種 (其中分別代表三種功能型式，增強者，沉默者與最少表現者的啟動子)

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.

如下圖所示，能表現 GUS (β-glucuronidase) 報導基因之 X 基因 植入不同的基因轉植植物 阿拉伯芥中，來代表上述的三種不同功能。當 *X* 基因被活化時，GUS 便能被表現出來。而 GUS 又可以將 MUS 轉化成 MU，此時利用螢光分光光度計檢測 MU 的螢光量便可得知 *X* 基因的活性。



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

帶有四種不同方式構築之報導基因的基因轉植阿拉伯芥

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.)

第一個實驗有兩種目的：(1) 找出含有 激素反應 ***cis*** **-** 單元的啟動子區域，(2) 研究不同濃度的激素 H 對 *X* 基因表現的影響。所有的轉植基因植物 (WT, dA, dAB 與 dABC) 已分別處理過濃度為 1μM 或 100 μM 的激素 H。並萃取植物萃取物用以評估 GUS 的表現量， (萃取物處理方式參考材料與方法的表格)

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.**

利用下節敘述的方法，分別測量 50μL 植物萃取物的 螢光吸光值 與 595 nm 的吸光值。根據測量的結果，分別計算每個萃取物中的 MU 的含量 (nmole MU/50μL 植物萃取液)，蛋白質濃度 (μg/50μL 植物萃取物) 與 GUS 活性 (nmole MU/μg protein/min)。並將結果記錄於答案紙的 <表一> 中，並依序回答 Q1.1, Q1.2 與 Q1.3。

Measurement of fluorescence and determination of MU amount

測量螢光與 MU 的含量

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

打開螢光分光光度計開關，取出 500 μL 標記為 GUS BL 的樣本進行 螢光 歸零。

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 the labeled WT-O and proceed in an order shown in the table in Materials and Equipment.

各取一支新的微量離心管，依序分別加入 50 μL 標記為 WT-O 【WT-O 為第一管，其他管順序則依表中順序添加】與 材料表格中 所列經激素處理過後的基因轉植植物萃取液，加入 50 μL 的 1 mM MUG 溶液。輕彈 離心管壁，均勻混合 上述兩種溶液。混合好的的樣本置於冰上保存，**直到所有樣本處理完畢後**，再進行下一步驟。

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

在 室溫下 培養上述混合樣本，反應時間為 10 分鐘，必須 **精準**。

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.

加入 **900 μL** 的終止液 (1M 碳酸鈉 在 GUS 萃取液中) 【WT-O 為第一管，其他管順序則依表中順序添加】。輕彈 離心管壁，均勻混合 上述兩種溶液。

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

分別自上述混合溶液中各取出 500μL，以螢光分光光度計測量 螢光含量。

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.

利用下列公式計算 MU 的含量。將螢光讀數記錄於答案紙中的 <表一>，並用它來計算 MU 的含量。每個數值分別代表 50 μL 植物萃取物所產生的 MU 量。

$$Y = 0.04\ X + 2.5$$

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

植物萃取液中 MU 的產生量 (nmoles 50μL 植物萃取液)

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

步驟 1)-5 中所測得的螢光量。

Measurement of absorbance at 595 nm and determination of protein amount

以 595 nm 吸光值 測量蛋白質含量

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

打開螢光分光光度計開關，取出 500 μL 標記為 Pro BL 的樣本進行 歸零。

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.

各取一支新的微量離心管，分別依序加入 50 μL 標記為 WT-O 或是 材料表格中 所列經激素處理過後的基因轉植植物萃取液，加入 950 μL 的 Bradford 溶液。輕彈 離心管壁，均勻混合 上述兩種溶液。將混合好的的樣本於室溫下反應 5 分鐘。

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

分別自上述混合溶液種各取出 500μL，以螢光分光光度計測量 595 nm 的吸光值。

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.

利用下列公式計算 蛋白質 的含量。將 595 nm 吸光值記錄於答案紙中的 <表一>，並用它來計算 蛋白質 的含量。每個數值分別代表 50 μL 植物萃取物蛋白質含量。

$Y = 98X + 2.8$

Y: the amount of protein (μg/50 μL plant extract)

混合物中 蛋白質 的含量 (μg/50 μL 植物萃取液)

X: the measured absorbance at 595 nm of the solution [from step 2)-3]

步驟 2)-3 中所測得的 595 nm 吸光值。

Calculation of GUS activity　　GUS 活性計算

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.

此酵素反應時間為 10 分鐘 (參考步驟 1)-3)。利用 <表一> 中所記錄的結果，計算 GUS 活性，單位為 nmole MU/μg protein/min。

Table 1 is worth of 9 points.

注意：<表一> 分數為 9 分。

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.

根據 <表一> 的結果，在 Q1.1 的表格中，正確處打勾 (√)。

Note: - stimulation: more than **3-fold** increase in gene *X* expression

注意：刺激反應：X 基因表現量達 3 倍或以上。

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

無反應：*X* 基因表現量 3 倍以下。

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.

根據 Q1.1 的結論，推論出 cis- 單元 (A~C)，分別隸屬於何種調控功能 (增強者，沉默者與最少表現者)。在 Q1.2 的表格中，正確處打勾 (√)。

Q1.3. (1 points)

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.

100 μM 濃度的 激素 H 處理下，*X* 基因的表現調控為何？根據 <表一> 的發現，決定 激素 H 的作用模式。在 Q1.3 的表格中，正確處打勾 (√)。

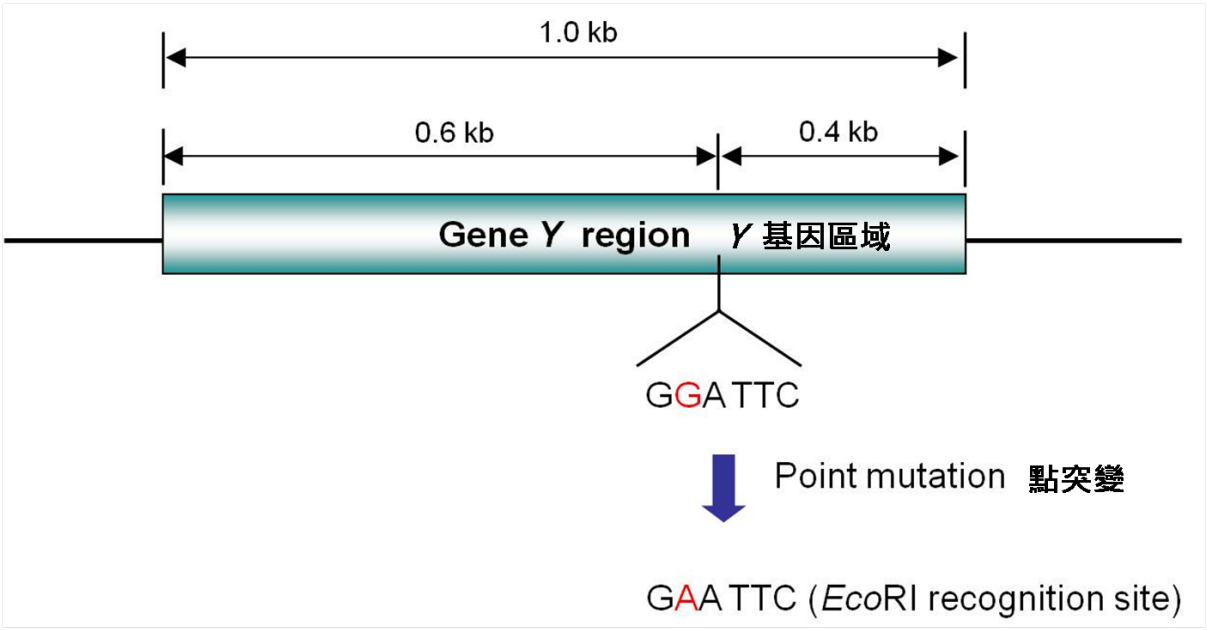
Part II. (15 points)

A co-relationship analysis between genotype and phenotype, and the prediction of gene pool frequencies using Hardy-Weinberg mathematics.

基因型與表現型相關分析，並利用哈溫定率計算基因池中的基因頻率。

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.

Y 基因會表現一轉蛋白調節植物生長。下圖為 基因體 DNA 中 Y 基因的區域，其中含有一個點突變。



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 recessive). 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.

現有 8 種可能為同型合子 (*YY* and *yy*) 或 異型合子 (*Yy*) 基因型之植物，分別表現出野生型與矮莖型之表型 (*Y*: 野生型對偶基因，*y*: 突變型對偶基因。並未特指出 Y 或 y 是顯性或隱性)。為了分析這些植物的基因型，利用 PCR 技術放大 Y 基因 1 kb 片段。這些片段分別以 *Eco*RI 限制酶切割，因為 *Eco*RI 會認識 GAATTC 序列並切割，且在此片段中並沒有起其它的 *Eco*RI 切點。利用下列步驟，進行電泳並分析 *Eco*RI 切割 PCR 產物的實驗。

Genotyping of gene *Y* by gel electrophoresis

利用電泳進行 Y 基因的基因型檢測

Note: Always wear polygloves during the experiment !!!

注意：全程都要戴上手套操作 !!!

(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.

全部共有 10 支樣本，分別裝在微量離心管中。兩支 DNA 大小片段標記管 (M)，8 支分別來自不同植物，並含有 *Eco*RI 處理過的 PCR 產物樣本 (分別標記為 P1~P8)。由 左邊 開始，依序加入樣本 **M, P1~P8, M**。每個樣本各取出 20 μL，利用固定刻度微量吸管，依照上面的順序，分別加入已經準備好的瓊脂膠體中的樣本槽中。**注意每個樣本都要換新的微量吸管頭。**

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.

注意：DNA 大小片段標記分別為 0.4, 0.6 與 1.0 kb。DNA 注入緩衝液與 DNA 染料已經在每個微量試管中添加完畢。

(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.

參考 DNA 電泳操作步驟，將蓋子裝上電泳槽，打開開關，開始進行電泳。

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

注意：電泳開始時，請注意 輸出 (output) 的指示燈是亮的，同時要有氣泡在白金電極處生成。

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

電泳時間為 30 分鐘，電壓為 HALF。

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

注意：電泳進行時，請回答第二大題 !!!

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

電泳完成後，關掉開關。舉起**綠色卡片**請求協助進行膠體拍照。

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

注意：助教會帶來一個膠體移轉盒，要確認你的 學生編號 要在盒子上。

(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.

當你收到你的膠體照片，利用膠帶將它貼在 答案紙 Q2.1 位置上。並將 P1~P8 的位置標示清楚。

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

在答案紙 Q2.2 有出現與 DNA 大小片段標記相同位置正確處打勾 (√)

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.

在答案紙 Q2.1 處貼上電泳照片。並標示出 P1~P8 的位置。

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.

分別確認每種植物 基因 *Y* 的 DNA 片段大小，與基因型 (*YY*, *Yy* 或 *yy*)。在答案紙 Q2.2 中正確處打勾 (√)。

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.

根據 Q2.2 基因型與表現型的說明，推論突變的特性。答案紙 Q2.3 中正確處打勾 (√)。

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.

將 植物 1 與 植物 3 進行雜交 (根據 Q2.2)，子代中矮莖的比例為若干？將正確答案填入答案紙中。

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.).

Q2.2 中的 8 種植物代表一種族群，如果這個族群產生 10,000 子代，則異型合子而且是矮莖的子代比例為若干？(前題是這個族群是在哈溫平衡狀態)

TASK II. (15 points)

Observation of meiotic cells in preserved rye anthers

觀察固定過的裸麥花粉囊中減數分裂細胞

Materials, instruments and tools **材料，器械與工具**	**Numbers** **數目**
1. Light microscope with objective lenses of 4X, 10X, 40X, and 100X 光學顯微鏡，物鏡倍率分別為 4X, 10X, 40X, 與 100X	1
2. Preserved rye anthers in a vial 裝有固定過的裸麥花粉囊小瓶	2
3. Dissecting needle set 探針組	1
4. Slides and cover slips 載玻片與蓋玻片	各 5
5. Filter paper (7 cm diameter) 直徑 7 公分濾紙	3
6. Forceps 鑷子	1
7. Ceramic tile 磁磚	1
8. Petri-dish (6 cm diameter) 6 公分培養皿	1

9. Acetocarmine solution with a dropper 醋酸洋紅溶液附滴管	1
10. Pencil 鉛筆	1
11. Eraser 橡皮擦	1
12. Disposable plastic pipet 拋棄式塑膠滴管	1
13. Red card 紅色卡片	1

Background 背景說明

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.

利用光學顯微鏡觀察裸麥花粉囊中的減數分裂細胞。花粉囊是減數分裂中的特定階段。花粉囊已經保存在 70% 酒精中。

Requirements – Overview

必需品 – 回顧

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**)

利用顯微鏡觀察減數分裂中的花粉細胞。利用 Q3.2 的範圍內，繪出在 **400X** 放大倍率下的減數分裂細胞。

Procedure 步驟

1) Before you start observation, check for the presence of two small preserved anthers in the vial.

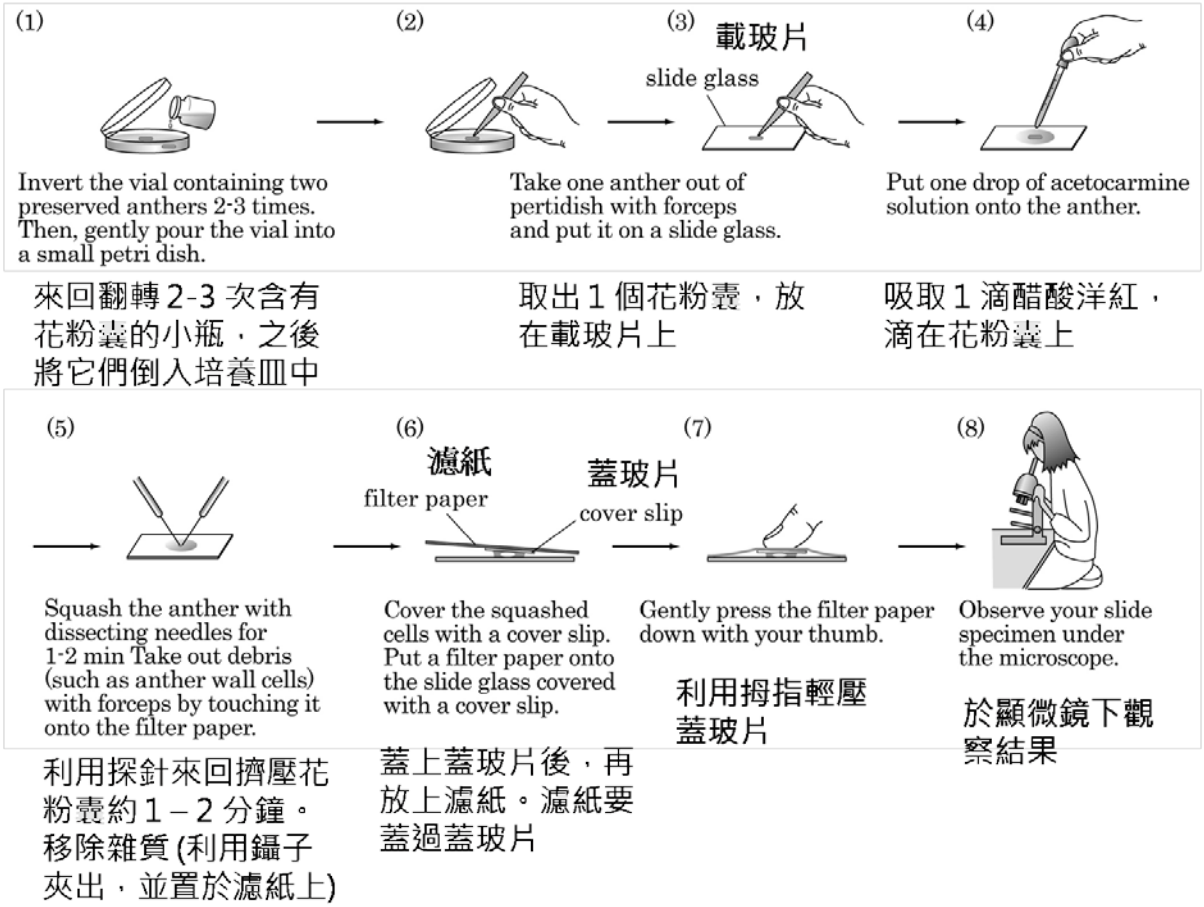
   實驗進行前，先確認瓶中裝有 **2 個** 花粉囊檢體。

2) Take out the ceramic tile out of the tray, and put one glass slide on it.

   自托盤中取出磁磚，將 載玻片 放在上方。

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 the drawing, raise the **red card**. The lab assistant will come to you and will take a photograph of the slide.

   在 100X 的放大倍率下，找到**至少 1 個** 正在進行減數分裂的細胞。將倍率換到 **400X**，觀察並繪製 **1 個** 減數分裂細胞，確認該細胞在視野的 **正中央**，觀察結果並繪製在 Q3.2 的範圍內。當你繪製完成後，舉起你的 **紅色卡片**，助教會過來幫你拍照。



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

觀察固定過的裸麥花粉囊中減數分裂細胞之實驗流程

Notes : 注意事項

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) .

   步驟 (1) 中，如果花粉囊沒有倒出來，將溶液裝回瓶中，利用拋棄式塑膠滴管將花粉囊吸出。

2. Be careful not to break the anther in step (2)

   注意，不要在步驟 (2) 時將花粉囊捏破。

3. You may use a filter paper to remove excess 70% ethanol in step (3).

   步驟 (3) 時可以使用濾紙將過多的 70% 酒精吸除。

4. Do not press too hard, or you may break the cells and/or the cover slip in step (7).

   步驟 (7) 時，不要壓得太猛，不然會將細胞壓破，不然就是壓破蓋玻片 。

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.

   每個人有兩個花粉囊，如果第一個沒有做的很好，得到好的影像，重複上述步驟再操作一次。**注意，你的實驗有時間限制。**

Q3. Answer the following questions.

回答下列問題。

*__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.

***特別注意：你將會在顯微鏡下看到兩種細胞型態，圈起來的是減數分裂細胞，其他的是花粉囊壁細胞。**

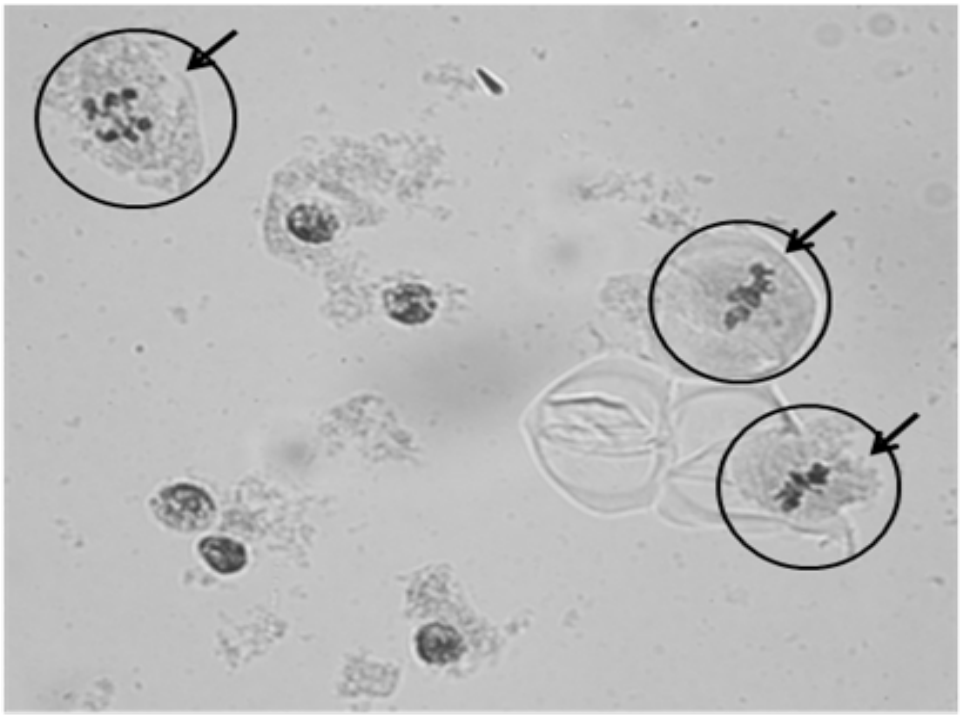


Figure Q3. Examples of cells undergoing meiotic cell division observed under a microscope.

圖 Q3：顯微鏡下觀察到的減數分裂細胞範例。

Q3.1. (1 point)

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

在花粉囊中哪些細胞會進行減數分裂？在答案紙中正確處打勾 (√)。

Q3.2. (8 points)

Draw **one cell** undergoing meiosis at 400X magnification in the answer sheet. Do not label the drawing.

將倍率換到 **400X**，觀察並繪製 **1 個** 減數分裂細胞，圖上不要做任何的標記。

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

重要事項：拍照時，必須確認該細胞在視野的 正中央。

Q3.3. (4 points)

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

你觀察到的細胞位於減數分裂週期的哪個階段？在答案紙中正確處打勾 (√)。

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.

你觀察到的細胞在減數分裂週期中，DNA 含量為若干？花粉囊壁細胞的 DNA 含量為若干？在答案紙中正確處打勾 (√)。