34th International Biology Olympiad
United Arab Emirates 2023

Plant Molecular Biology 植物分子生物

34th International Biology Olympiad

3-10 July 2023, United Arab Emirates University

Practical Exam

實作考試

Plant Molecular Biology

植物分子生物學

Total points 總分: 100

Duration 考試時間: 90 minutes 分鐘

General Instructions:

In this practical exam, you have 90 minutes to complete **TWO tasks.**

在本實作考試中，你在 90 分鐘內要完成 2 組實驗試題 You can perform the experiments in any order.

你可以自訂實驗試題的進行順序

- **Task 1: Restriction digestion of DNA, and its analysis (65 points)**
- **Task 2: Plasmolysis of onion cells and its analysis (35 points)**

第 1 題:DNA 的限制酶切割及其分析 (65 分)

第 2 題: 洋蔥細胞的原生質離及其分析 (35 分)

During task 1 there is time for incubation and gel run. Use this time to carry out any task of your choice.

在第一題中有樣品靜置時間和電泳時間，利用這些時間去進行其他的實驗題。

Important Information:

重要訊息：**Write your answers in the answer sheet. Only answers given in the answer sheet will be evaluated.**

把答案寫在答案紙上，只有在答案紙上的答案才會被評分

Make sure that you have received all the materials and equipment listed, including a graph paper. If any of these items are missing, please raise your card immediately.

請確定你有所有的材料及器材，包括作圖方格紙。若有任何缺少項目，請立即舉卡通知監考人員。

During experiments, ensure that you wear gloves, and handle the equipment and samples carefully.

操作實驗時，要戴手套並小心操作儀器和樣品。

Any spilled solutions, samples or equipment damaged by you will not be replaced, apart from gloves.

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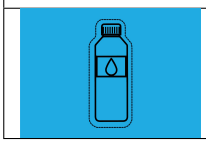
Q1-2

Tranditional Chinese (Chinese Taipei (Taiwan))

任何潑灑掉的溶液、樣品，或是儀器被你損壞，都將不會補充或替換，除了手套。

Use the following cards to ask for water/washroom/incubation/photography/queries.

若需要飲水/洗手間/協助，請舉下列卡片

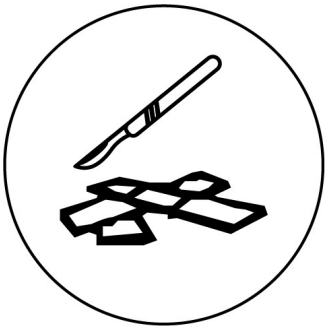
Drinking water 飲水	Washroom 洗手間	Sample incubation 樣品恆溫靜置	Photographic documentation 照相	Other queries 其他需求
				

IMPORTANT: Two waste bins have been provided on the desk

重要: 桌上有提供兩個廢棄物桶

- DISCARD COVERSLIPS, SLIDES AND SCALPEL IN THE WASTEBIN MARKED

把使用過的蓋玻片、載玻片和解剖刀片丟棄在指定的廢棄物桶中



- DISCARD BIOLOGICAL MATERIAL SOLUTIONS ETC. IN THE WASTEBIN MARKED
- 把生物材料、溶液等丟棄至指定的廢棄物桶中

Stop answering as soon as you hear the whistle at the end of the exam.

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Q1-3

Tranditional Chinese (Chinese Taipei (Taiwan))

在實作結束之鈴聲響起，應立即停止作答

No paper, materials or equipment should be taken out of the laboratory.

不能把紙張、材料或儀器帶出實驗室

Good luck!

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Q1-4

Tranditional Chinese (Chinese Taipei (Taiwan))

Materials provided for task 1:

第 1 題的材料

1. 50 µl of linear DNA (50 ng/µl) labelled **DNA**; on ice. 50 µl 的直線型 DNA (50 ng/µl)，標示「DNA」；在冰上
2. 30 µl of HindIII restriction enzyme labelled **H3,** on ice. 30 µl HindIII 限制酶，標示「**H3**」；在冰上
3. 30 µl of Kpn I restriction enzyme labelled **KI,** on ice. 30 µl KpnI 限制酶，標示「**K1**」；在冰上
4. 20 µl of 10X buffer for restriction digestion labelled **BUF,** on ice. 20 µl X 限制酶反應緩衝液，標示「**BUE**」；在冰上
5. 100 µl of nuclease free water labelled **W**; on ice. 100 µl 無核酸酶水，標示「**W**」；在冰上
6. 30 µl of 6X loading dye labelled **LD**; on ice. 30 µl 6X 注膠染劑，標示「**LD**」；在冰中
7. 2 –20 µl Micropipette 2 –20 µl 微量分注器
8. 1 box of yellow tips. 1 盒黃色微量吸管尖
9. 4 x 1.5 ml microfuge tubes labelled as 1, 2, 3, and 4; on stand (labelled with desk number) 4 支 1.5 ml 微量離心管，標示 1, 2, 3, 和 4; 在離心管架 (有標示桌號) 上
10. Agarose gel electrophoresis system with incorporated power supply 瓊脂膠電泳裝置，附電力供應器
11. Agarose gel containing DNA binding stain (already placed in the electrophoresis system). 瓊脂膠 (內含 DNA 染劑，已置放電泳裝置內)
12. Photograph of digestion pattern corresponding to Figure 1 and 3 in the write up. 對應圖 1 和圖 3 切割電泳結果的照片
13. Ruler 尺

Materials for task 2:

1. Scales of peeled onion (in plastic bag) 洋蔥鱗葉 (在塑膠袋中)
2. Slides (in plastic bag) 載玻片 (在塑膠袋中)
3. Coverslips (in small petridishes) 蓋玻片 (在小培養皿中)
4. Fine forceps (x 2) 細鑷子 (2 支)
5. Fine needle (x 1) 細探針 (1 支)
6. Scalpel (x 1) 解剖刀 (1 支)
7. Brush (x 1) 細水彩筆 (1 支)
8. A cardboard cup containing 15 ml tubes each with approximately 6 ml of: 紙杯中裝有數支 15 ml 試管，每管大約有 6 ml 的以下溶液或器材

- Solution isotonic to onion cells (labelled **IS**) 洋蔥細胞的等張溶液 (標示為 **IS**)
- Deionized water (labelled **W**) 去離子水 (標示為 **W**)
- 20% NaCl solution (labelled **20N**) 20% NaCl 溶液 (標示為 **20N**)
- Solution labelled **A 溶液 A**
- Solution labelled **B 溶液 B**
- Solution labelled **C 溶液 C**

- Plastic droppers (x10) 塑膠滴管 (10 支)

9. Microscope 顯微鏡
10. Photograph of onion epidermal cells corresponding to Figure 4. 對應圖 4 的洋蔥表皮細胞之照片圖

Material common to both tasks:

1. Orange card with sign of thermometer (temp. flag). 有溫度計圖樣的橘色卡 (temp. flag)
2. Green card with sign of camera (photo flag). 有相機圖樣的綠色卡 (photo flag)
3. Red card with sign of "**?**" (general query). 有？圖示的紅色卡 (一般詢問)
4. Yellow card with a sign for washroom (toilet break). 有洗手間圖示的黃色卡 (上廁所)
5. Blue card with a sign of water bottle (drinking water). 有水瓶圖示的藍色卡 (喝水)
6. Digital Clock. 數字鐘
7. Tissue paper. 衛生紙
8. Waste bins (x2) 廢棄物桶 2 個
9. Disposable gloves. 拋棄式手套

Task 1. Restriction digestion of DNA and its analysis 第 1 題 DNA 的限制酶切割和分析

INTRODUCTION

背景介紹

Digestion of DNA with restriction enzymes creates fragments of different sizes. The DNA fragments generated by digestion can be resolved based on their size, by agarose gel electrophoresis.

以限制酶切割 DNA 會產生不同大小的片段。這些片段可以依據他們大小用瓊脂膠電泳將他們分離解析。

First, you are required to set up a restriction digestion of linear DNA (provided) with enzymes HindIII (H3) and KpnI (KI), and analyze the resulting DNA fragments by agarose gel electrophoresis. The DNA fragments will be visualized using GelGreen stain (non-hazardous), which fluoresces under blue light (non UV) when bound to DNA. As an analytical task, you will have to assess the size of some of the DNA fragments.

首先，你要以限制酶 HindIII (H3) 和 KpnI (K1) 切割一條直線型 DNA，然後將切割所得的各 DNA 片段用瓊脂膠電泳將他們分離解析。這些電泳 DNA 片段，將以無毒的 GelGreen 染色，當這種染劑結合到 DNA 後，在藍光 (非 UV) 照射下會發出螢光。在此題的分析部分，你需要計算一些 DNA 片段的大小。

In a second analytical task, you will be analyzing a photograph of restriction digestion profile of a plasmid (vector) originally constructed for carrying out plant transformation. Scientists routinely check whether vectors are assembled correctly by carrying out restriction digestions, which could then be followed by sequencing of different parts of the vector.

此題的第二部分，你要對一個質體 (載體) 的限制酶切割結果照片進行分析，此質體是為了進行植物轉形實驗而建構。科學家習慣先以限制酶切割檢測載體是否正確組合，之後可以再對載體的各部分進行定序。

Part 1 第 1 部分:

1.1. Restriction digestion of DNA (32 Points) 1.1 DNA 的限制酶切割 (32 分)

PROCEDURE 步驟

1. Set up the restriction digestion reactions in the 1.5 ml microfuge tubes labeled 1, 2, 3, and 4. Add the components into each tube serially, as mentioned in Table 1.1.

1. 在標示 1, 2, 3, 和 4 的 1.5 ml 的微量離心管中調配限制酶切割反應液，依據表 1.1 的配方在每一個微量離心管中分別加入指定的成分。

S. No.	Component 內容物	Tube 1	Tube 2	Tube 3	Tube 4
	Volume (µl) 體積				
1	Nuclease free water 無核酸酶水 (W)	13	8	8	3
2	Linear DNA 直線型 DNA (DNA)	5	5	5	5
3	Restriction Buffer 限制酶反應緩衝液 (BUF)	2	2	2	2
4	Hind III 限制酶 (H3)	0	5	0	5
5	Kpn I 限制酶 (KI)	0	0	5	5
	Total volume 總體積 (µl)	20	20	20	20

Table 1.1

2. Make sure that the components are mixed well by using the micropipette.

2. 用微量分注器將管中成分確保混合均勻。

3. Place the tubes in the microfuge stand (check whether the number on the rack corresponds to your table number).

3. 將離心管放在離心管架中，檢查離心管架上號碼是否和你的實驗桌號相符。

4. Call the scientific volunteer immediately, by raising the‘temp flag’. The scientific volunteer will place the tubes in an incubator at 37^0C for 20 minutes. Record the start and stop time.

4. 立即舉起”temp flag”，請現場科學志工將離心管放到 37˚C 恆溫箱中進行反應 20 分鐘，記下反應開始的時間和反應結束的時間。

Start time 開始時間**:**

Stop time 結束時間 **:**

5. After 20 minutes, raise the ‘temp flag’ to ask the scientific volunteer to get your tubes from the incubator.

5. 20 分鐘後，舉起”temp flag”，請現場科學志工將離心管自恆溫箱中取回。

6. Add 4µl of loading dye (LD) into each of the 4 tubes. Mix by using the micropipette.

6. 分別將 4 ml 注膠染劑 (LD) 加入各離心管中，用微量分注器混合均勻。

7. Get ready to load the gel. Leave the gel electrophoresis apparatus turned off at this time. In the upper right corner, there are two buttons for high-level or low-level blue illumination. Switch on the low-level blue illumination. This will enable you to see the wells clearly. Be careful **not to** press the power button. Do not move the gel electrophoresis apparatus.

7. 準備樣品注膠，將電泳裝置右下的電泳電源按鍵維持在關閉狀態，在裝置右上角有二個按鍵分別控制強藍光和弱藍光，開啟弱藍光照明，可以讓你看清楚樣品孔。小心不要按右下的電泳電源按鍵，不要移動電泳裝置。

8. Leave the first well blank. Load 20 µl of each sample from tube numbers 1 to 4 consecutively into 4 separate adjacent wells of the electrophoresis gel (left to right, when positive pole is closer to you).

NOTE: The gel is covered with electrophoresis buffer; so, load the samples very gently inside each well to prevent spillover while loading.

8. 略過第一個樣品孔，分別將離心管 1 到 4 號中 20 µl 的樣品注入電泳膠中接續相鄰的的 4 個樣品孔 (自左至右，正極側靠近你)。

注意：因為電泳膠被緩衝液覆蓋，所以請非常輕巧地將樣品注入樣品孔中，不要讓樣品溢出。

9. After you have finished loading the samples, place the orange-colored photo hood onto the apparatus. Switch off the low-level illumination.

9. 完成樣品注入後，將橘色照相罩子放回電泳裝置上，關掉弱藍光照明。

10. Run the gel for 40 minutes. Start the electrophoresis unit by pressing the power button on the apparatus' lower right corner. Record the start and stop time. To keep track of the time, you can note the start time and calculate the expected stop time.

10. 電泳時間 40 分鐘，按下右下角電泳電源按鍵，開始進行電泳。你可以從開始電泳的時間去計算預期停止電泳的時間。記下電泳開始和結束的時間

Start time 開始時間 **:**

Stop time 結束時間 **:**

11. Photo hoods should not be removed during the run. Stop the run by pressing the power button.

11. 電泳進行時，不要移開照相罩子。要停止電泳時，按下電泳電源按鍵。

12. Press the button for high-level lighting. Due to the DNA binding stain in the gel, you will be able to see DNA through the hole on top of the photo hood.

12. 按下強藍光照明，因為 DNA 染劑已在電泳膠中，你可以從照相罩子上方的圓孔看到 DNA。

13. Raise the ‘photo flag’ to call the scientific volunteer. The scientific volunteer will take the photograph.

13. 舉起”photo flag”，請科學志工協助拍照。

14. Check and approve the photograph. It will be printed and given to you with your student code written on it. Submit the photograph with the answer sheet.

14. 檢視並確認照相結果後，會印出相片，其上有你的學生編號，將照片和答案卷一起繳交。

Q.1.1.1 Attach the photograph of the gel 貼上電泳結果照片	32.0pt

Part 2 第 2 部分:

1.2 DNA fragment analysis: Sizing digested DNA (24 Points)

1.2 DNA 片段分析：估算切割後 DNA 片段的大小 (24 分)

The picture in **Figure 1** represents the profile of fragments (**Tubes 1 to 4**) observed following a digestion with the same combination of restriction enzymes (in the same order) as in part 1. Reaction time and enzyme activity were sufficient to allow for a complete digestion of the DNA. To determine the sizes of different fragments, the gel was run for a longer period of time. Fragments smaller than approximately 1000 base pairs (bp) are not observed in the gel. For the analysis, a photograph of the same gel has been provided in addition to figure 1.

圖 1 中的照片是前述 DNA 經過和第 1 部分相同的限制酶切割組合 (相同的順序) 和電泳後所得的 DNA 片段結果圖 (管 1 到管 4)。其反應時間和酵素活性均足夠完全切割 DNA，且為了判定各片段的大小，電泳進行時間較長。此電泳膠上看不到小於 1000 鹼基對 (bp) 的片段。為分析目的，此圖 1 會提供另附的清晰照片。

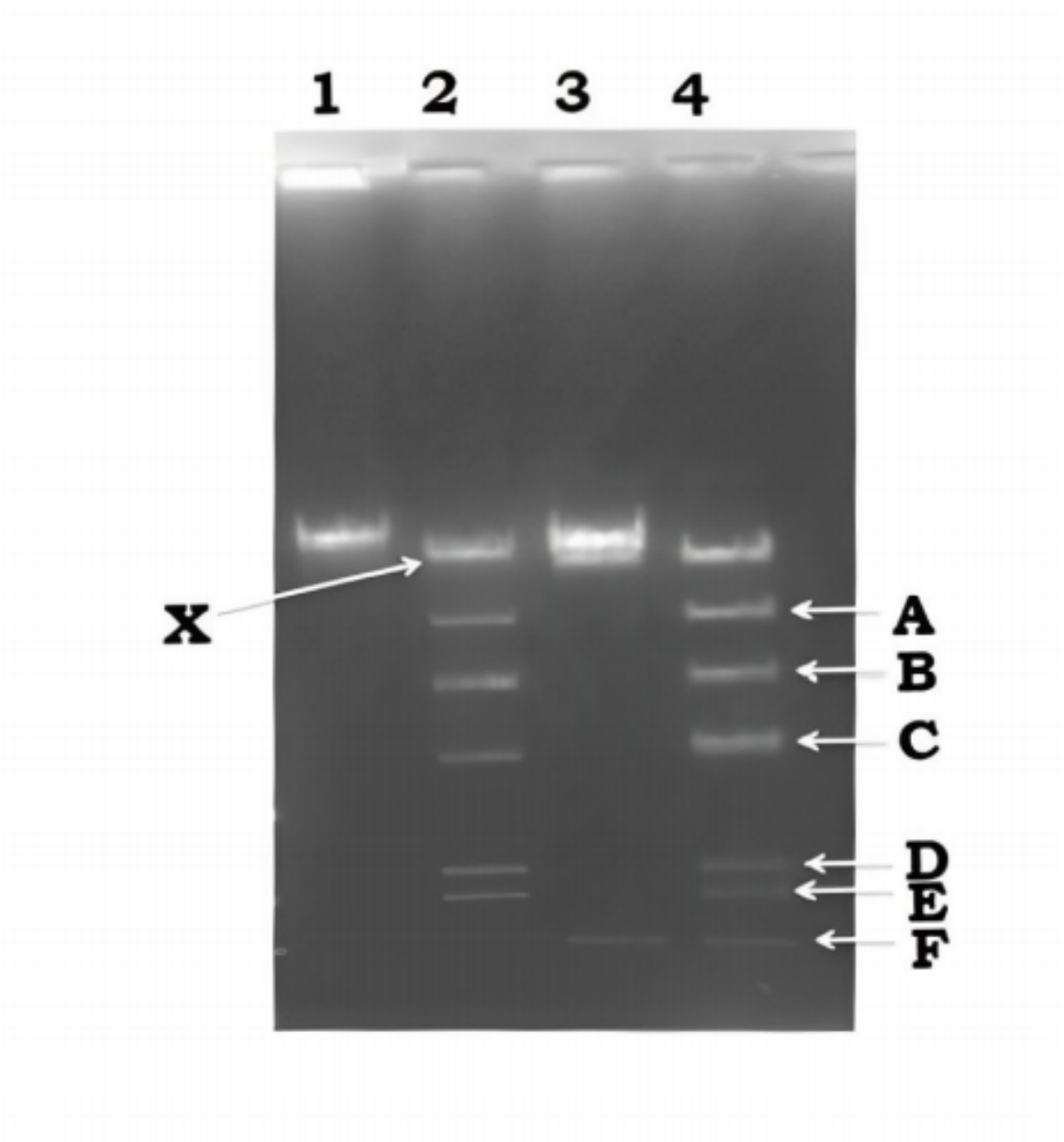


Figure 1. Digestion profiles of linear DNA run on agarose gel.

圖 1. 直線型 DNA 經限制酶切割後的瓊脂膠電泳結果圖

The size of DNA fragments generated by restriction digestion, can be determined by comparing their migration, on agarose gels, with those of DNA fragments of known sizes (molecular weight or size markers).

經限制酶切割後的 DNA 片段大小，可以藉比對已知分子量的 DNA 標準品片段在瓊脂膠電泳的移動而估算得知。

Following gel electrophoresis, the migration distance of each fragment of the size marker is measured. The rate at which a DNA fragment travels during electrophoresis is inversely proportional to the log_{10} of

its length in base pairs.

電泳後，量測每一個分子量 DNA 標準品片段在膠上的移動距離。DNA 片段在電泳時的移動速率和其鹼基對長度的log_{10} 成反比。

The distance migrated can be plotted against the log_{10} of fragment length. The graph generated by the size marker will contain a region represented by a straight line. The size of the unknown fragments can then be determined using this plot.

DNA 片段在電泳膠上的移動距離，和其長度的log_{10} 值可以畫成對應關係圖。在以已知分子量 DNA 標準品片段的電泳距離畫出的關係圖中，會有一個直線區域，未知片段的大小可利用此關係圖推定。

In this exercise you are required to calculate the size (bp) of the DNA fragments C and E.

在以下試題中，你要計算 DNA 片段 C 和 E 的鹼基對 (bp) 大小。

1. The distance migrated (cm) by fragments A to F has been given in Column II of Table 1.2 表 1.2 的第 II 行是片段 A 到 F 在電泳膠上的移動距離 (cm)。
2. Table 1.2 (Column III) provides the size of DNA fragments (A, B, D, and F). 表 1.2 的第 III 行是 DNA 片段 A、B、D 和 F 的已知大小。
3. Calculate the log_{10} value of the size of fragments A, B, D and F. Record the log_{10} values, rounded to 2 decimal places, in column IV. 計算片段 A、B、D 和 F 的大小 log_{10} 值，四捨五入至小數點下 2 位，紀錄於表 1.2 的第 IV 行。
4. Plot a graph of distance migrated against log_{10} size in the graph paper provided along with the answer booklet. 在答案卷的作圖方格紙上畫出電泳移動距離和鹼基 log_{10} 值大小的對應關係圖。
5. Use an appropriate range for the X and Y axes. 選用適合的 X 和 Y 軸的區間。
6. The plot should be scaled appropriately. 作圖須標示適宜的間隔和單位
7. Choose the suitable roman numbers (I to VI) from Table 1.2 to label the X and Y axes. 自表 1.2 中的羅馬數字 (I 到 VI)，選擇正確的數字對應標示在 X 和 Y 軸。
8. Plot a line of best-fit. 在圖中畫出最佳擬合線。
9. Based on the best-fit line, record the log_{10} size, to two decimal places of fragments C and E in column V of Table 1.2. 依據最佳擬合線，找出片段 C 和 E 的 log_{10} 值大小至小數點下 2 位，紀錄於表 1.2 的第 V 行。
10. Calculate the sizes of fragments C and E and record in column VI of Table1.2, rounded to the nearest whole number. 計算片段 C 和 E 的鹼基對大小，取至最接近的整數，紀錄於表 1.2 的第 VI 行。

Q.1.2.1 Plot the graph 畫出對應關係圖	6pt

Q.1.2.2 Fill table 1.2 完成表 1.2 14pt

Table 1.2 表 1.2

I	II	III	IV	V	VI
DNA fragment DNA 片段	**Distance migrated 移動距離 (cm)**	**Size 鹼基對大小 (bp)**	Log_{10} **size** Log_{10} **大小 (off to 2 decimal** 小數點下 2 位**)**	Log_{10} **size for fragment C and E 片段 C 和 E 的** Log_{10} **大小 (from graph 由圖中求得)**	**Size (bp) fragments C and E 片段 C 和 E 的鹼基對大小 (bp)**
A	5.3	9416		×	×
B	6.0	6557		×	×
C	6.9	×	×		
D	8.2	2322		×	×
E	8.5	×	×		
F	9.1	1514		×	×

Q.1.2.3 Mark a cross (X) in the appropriate column. 4.0pt
Q.1.2.3 在適當的空格標示選擇符號 (X)

Statement 敘述	True 對	False 錯
Kpn I digestion, generates 3 DNA bands. Kpn I 酵素切割後產生 3 個 DNA 片段條帶		
There are three sites targeted by Kpn I in the linear DNA provided 在此直線型 DNA 上有 3 個 Kpn I 切位		
All sites targeted by Kpn I are present in the DNA band marked 'X' in lane 2. 所有的 Kpn I 切位都在電泳圖第 2 lane 中標示’X’的 DNA 片段條帶。		
If band B contains 2 pmoles of DNA strands, band E will also contain 2 pmoles of DNA strands. 若 DNA 片段條帶 B 含 2 pmoles 皮莫耳的 DNA，則 DNA 片段條帶 E 也會含 2 pmoles 皮莫耳的 DNA		

Part 3:

1.3 Generating a restriction map. (9 points)

1.3 推定限制酶切位圖譜 (9 分)

Figure 2 represents a partial restriction map of the plasmid vector (pACE2023) used for plant transfor-

mation. pACE2023 is 16500 base pairs (bp) in size.

圖 2 是質體載體 pACE2023 限制酶切位圖譜，pACE2023 使用於植物轉形實驗，pACE2023 其大小為 16500 bp。

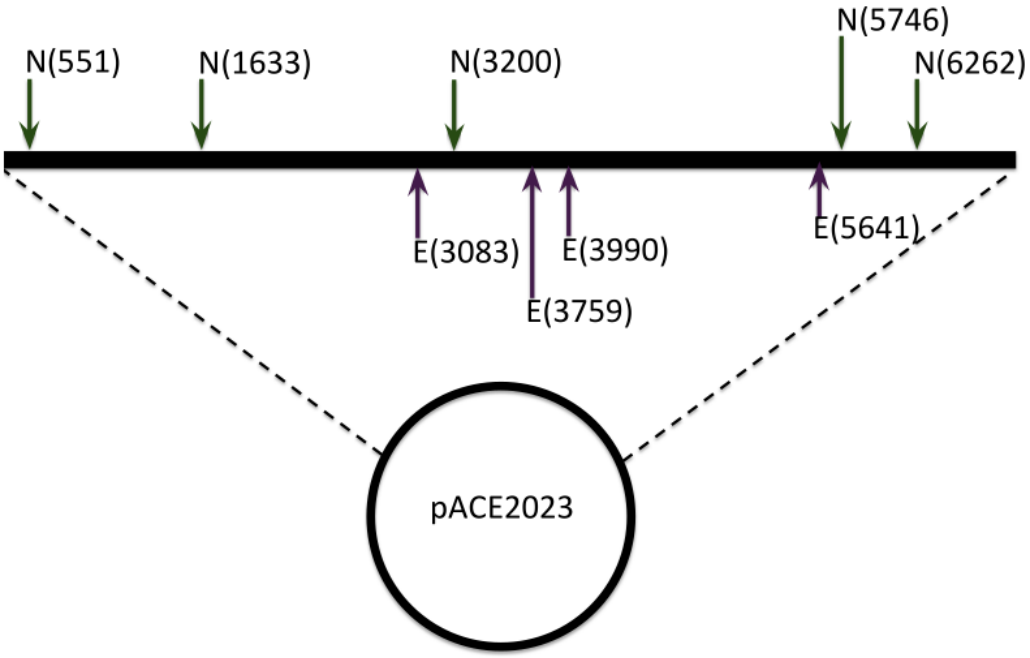


Figure 2: Map of pACE2023. The positions of different restriction enzyme targeting sites have been shown. N= NcoI; E= EcoRV. The numbers in brackets represent the position of that restriction site (in base-pairs).

圖 2：pACE2023 限制酶切位圖。

圖中標示不同限制酶的切位圖，N=NcoI；E=EcoRV。括號中的數字代表此限制酶切位的位置(鹼基對序位)。

All sites for NcoI and EcoRV have been shown.

所有的 NcoI 和 EcoRV 切位都已標示

pACE2023 was digested with different restriction enzymes, the result of which is presented in Figure 3.

pACE2023 以不同限制酶切割後的電泳分析結果為圖 3。

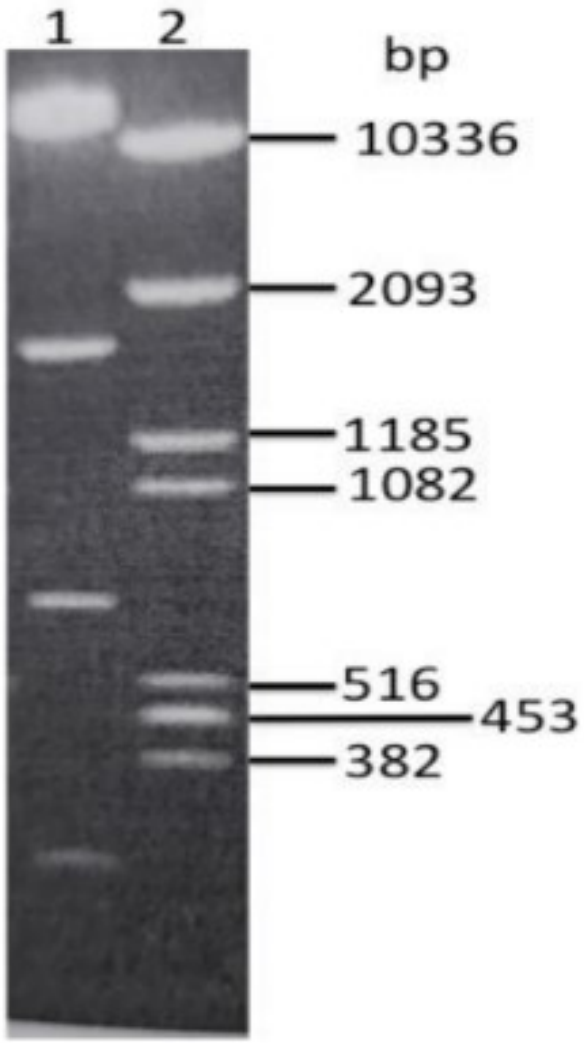


Figure 3: Digestion profile of pACE2023. Lane 1: vector digested with EcoRV (E); lane 2: vector double digested with both HindIII (H) and NcoI (N).

圖 3：pACE2023 限制酶切割結果圖。

第 1 lane：載體以 EcoRV (E) 切割；

第 2 lane：載體同時以 HindIII (H) 和 NcoI (N) 切割。

The size of DNA bands in lane 2 is indicated.

第 2 lane DNA 片段條帶的大小標示於右側。

The intensity of fluorescence of the 453 bp fragment is double that of the 516 bp fragment.

圖中 453 bp 條帶的螢光強度是 516 bp 條帶的螢光強度的 2 倍。

Answer the following questions:

回答以下問題：

Q.1.3.1 What is the size (in base pairs) of the largest fragment obtained upon digestion with EcoRV? 1.5pt
以 EcoRV 切割後，所得最大片段的鹼基對大小是多少？

Q.1.3.2 How many Hind III targeted sites are present in pACE2023? 2.5pt
質體 pACE2023 上有多少 HindIII 切位？

Q.1.3.3 Identify the location of the Hind III site(s) in pACE2023 by marking a cross (X) in the appropriate cell(s) (one cross for each site) in the table . Mark a circle in the remaining cell(s). 5.0pt
推定 pACE2023 上 HindIII 切位的位置，在表中正確項空格標示選擇符號 (X)。其他空格中則標示一個圓圈 O。

Outside 551 and 6262 region 在 551 和 6262 區間之外	Between 551 and 1633 在 551 和 1633 之間	Between 1633 and 3200 在 1633 和 3200 之間	Between 3200 and 5746 在 3200 和 5746 之間	Between 5746 and 6262 在 5746 和 6262 之間

Task 2. Plasmolysis of onion cells –an analysis 洋蔥細胞的原生質離及其分析

INTRODUCTION 前言

Water potential is defined as the difference of free energy present in the water of a system when compared to free energy of pure water at constant temperature and pressure. It influences the direction of movement of water in plants.

水勢的定義是：當溫度和壓力恆定時，某系統中水的自由能 (free energy) 與純水自由能的差異。水勢會影響水在植物體內的移動方向。

The symbol for water potential is

ψ (psi). 水勢的符號是 ψ (psi)

Potential is measured in units of pressure, usually in megapascals (MPa).

水勢測量單位是壓力，通常是百萬帕斯卡 (MPa)

In living cells, water potential can be calculated by the equation

在活的植物細胞中，水勢的計算公式是

$\psi = \psi p + \psi s$

where,

1. ψp represents the pressure potential (turgor pressure, i.e., pressure of protoplast against cell wall).
2. ψs is osmotic potential or solute potential; the effect that solutes have on water potential. Pure water contains no solutes and has a ψs of 0.0 MPa.

其中

1. ψp 代表壓力勢 (膨壓，亦即原生質壓向細胞壁上的壓力)

2. ψs 代表滲透勢或溶質勢；亦即溶質對水勢的作用，純水因為沒有溶質，所以其 ψs = 0

In this experiment, you will observe the processes of plasmolysis and deplasmolysis in epidermal cells of onion. Based on this, you are required to identify the percentage of NaCl in the three given solutions labelled A, B and C.

在本實驗中，你將觀察洋蔥表皮細胞”原生質離”以及”原生質回復”的過程。根據此過程，你必須判斷三種溶液 (分別標示為 A, B, C) 的 NaCl 氯化鈉百分比濃度

Part 1: 第 1 部分

2.1 The first task involves mounting onion epidermal cells in a solution that will not affect their turgidity. Carry out the following steps:

第 1 部分是要先將洋蔥表皮細胞浸置於不會影響其膨壓的溶液中，其操作步驟如下:

1. Choose the appropriate solution for the experiment. Record the solution used in **Table 2.1.** 選擇適合加入此實驗的溶液，在表 2.1 中記錄下結果
2. Place 2-3 drops of the solution on a slide. 滴加你選的溶液 2-3 滴在載玻片上
3. Choose an onion scale from the middle layers. With the help of the scalpel, cut out a piece of the onion scale of approximately 1cm X 1 cm. 從洋蔥中間部位的幾層中選一鱗葉，利用解剖刀切下約 1cm X 1 cm 的鱗葉。
4. With the help of forceps peel off the epidermal layer from the inner side of the scale. 使用鑷子撕下鱗葉內層的表皮

5. Spread the epidermal layer in the solution on the slide. solution on the slide. 把表皮放在載玻片的溶液中並攤平
6. Make sure that the peel is a flat single layer of cells and is completely immersed. Keep it immersed with the help of a brush. 確定所撕下的表皮只有一層細胞厚度，且完全浸在溶液中，可用細水彩筆讓表皮完全浸入
7. Carefully place a coverslip over the peel so that there are no air bubbles. 小心地蓋上蓋玻片在表皮上方，避免氣泡產生
8. Wipe off the extra fluid from outside the coverslip with tissue paper. 用衛生紙擦乾蓋玻片周圍多餘的溶液
9. Switch on the microscope, using the switch on the upper left. This switch is marked with I,II and III. Switch to III for best illumination. Do not touch the wheel marked 8 at the bottom left. 打開顯微鏡左上方的開關，此開關有三段，第三段最亮，請勿觸摸左下角標記為 8 的旋轉鈕
10. Observe the slide at 100X magnification. Note: The magnification power of the eyepiece is 10X. Choose the appropriate objective lens to achieve a total magnification of 100X. 在 100X 下觀察所製成的玻片，注意: 目鏡的倍率是 10X，選擇適當的物鏡以達到總放大倍率為 100X
11. Record the magnification power of the objective lens you choose in **Table 2.1.** 在表 2.1 中記錄所使用的物鏡倍率
12. Focus on a field in the slide that is an appropriate representation of your observation. 選擇一適當的視野來對焦並觀察
13. Match your observation to the panel in **Figure 4**. A photograph of the same panel has been provided in addition to **Figure 4**. 將你的觀察結果對應到圖 4 圖片樣版中適當的圖號。此外，會提供和圖 4 相同的照片
14. Record which one of the panels (4a to i) is closest to your observation by marking a cross (X) in the appropriate cell of **Table 2.1. Mark only one cell.** 記錄圖片樣版中的圖號 (4a to i) 最接近你的觀察，並在表 2.1 中的適當空格中標記 X，只標記一個空格
15. Raise the ‘photo card’ to call the scientific volunteer. The scientific volunteer will take photographs of the field you have focused on. 舉起‘photo card’的牌子，提醒監考人員來幫你拍照
16. Check and approve the photograph. It will be printed and given to you with your student code written on it. Submit the photograph along with the answer sheets. 檢視並確定拍照結果。這照片將被印出來並註記你的學生編號。你必須把照片和答案紙一起繳回。

Q.2.1	Attach the photograph 附有照片圖	10.0pt

2.2 In this part, you are required to observe the structure of the epidermal cells in 20% NaCl.

此實驗中，你須觀察在 20% NaCl 下的表皮細胞構造

1. Place 2-3 drops of 20% NaCl solution on a slide. 將 20% NaCl 溶液 2-3 滴，滴在載玻片上
2. Carry out the steps mentioned in 2.1.3 to 2.1.6. 依照上述 2.1.3 to 2.1.6 步驟操作
3. Incubate in 20% NaCl for 5 minutes. 在 20% NaCl 中浸置 5 分鐘
4. Observe the preparation at a magnification of 100X. 在 100X 下觀察所製玻片
5. Carry out the steps mentioned in 2.1.12 and 2.1.13. 操作上述步驟 2.1.12 和 2.1.13
6. Record which one of the panels (4a cellsto i) is closest to your observation by marking a cross (X) in the appropriate cell of Table 2.1. Mark only one cell. 在最接近你的觀察之圖片樣版 (4a cellsto i) ，並用 X 在表 2.1 的適當空格中作記錄。只標記一個空格。

2.3 In this part, you are required to observe the structure of the epidermal cells after incubation in 20% NaCl for 5 minutes followed by incubation in **Solution A for 5 min.**

此部分的實驗中，你必須觀察表皮細胞在 20% NaCl 中浸置 5 分鐘之後，再以溶液 A 處理 5 分鐘後的構造變化

1. Carry out the steps as mentioned 2.2.1 to 2.2.3. 依照上述 2.2.1 to 2.2.3 步驟操作
2. With the help of a dropper, remove the 20% NaCl solution and immerse the peel in Solution A. Incubate for 5 min. 使用吸管吸掉 20% NaCl 溶液，然後浸置在溶液 A 中，處理 5 分鐘
3. Observe the preparation at a magnification of 100 X. 在 100X 下觀察所製玻片
4. Carry out the steps mentioned in 2.1.12 and 2.1.13. 操作上述步驟 2.1.12 和 2.1.13
5. Record which one of the panels (4a to i) is closest to your observation by marking a cross (X) in the appropriate cell of **Table 2.1. Mark only one cell.** 記錄圖片樣版中的圖號 (4a to i) 最接近你的觀察，並在表 2.1 中的適當空格中標記 X，只標記一個空格。

2.4 Carry out the steps as mentioned above in 2.3 to record your observation when the epidermal peel is incubated in 20% NaCl for 5 minutes, followed by incubation in **Solution B for 5 min.**

依照上述 2.2.3 步驟操作，觀察、記錄表皮細胞在 20% NaCl 中浸置 5 分鐘之後，再以溶液 B 處理 5 分鐘後的構造變化

Record which one of the panels (4a to i) is closest to your observation by marking a cross (X) in the appropriate cell of **Table 2.1. Mark only one cell.** 記錄圖片樣版中的圖號 (4a to i) 最接近你的觀察，並在表 2.1 中的適當空格中標記 X，只標記一個空格。

2.5 Carry out the steps as mentioned above in 2.3 to record your observation when the epidermal peel is incubated in 20% NaCl for 5 minutes, followed by incubation in **Solution C for 5 min.**

依照上述 2.2.3 步驟操作，觀察、記錄表皮細胞在 20% NaCl 中浸置 5 分鐘之後，再以溶液 C 處理 5 分鐘後的構造變化

Record which one of the panels (4a to i) is closest to your observation by marking a cross (X) in the appropriate cell of **Table 2.1. Mark only one cell.** 記錄圖片樣版中的圖號 (4a to i) 最接近你的觀察，並在表 2.1 中的適當空格中標記 X，只標記一個空格。

Q.2.2 Fill in Table **2.1** 15.0pt
填寫表 2.1

Table 2.1									
2.1.1. Solution used for task 2.1 = 在 2.1 題所使用的 **2.1.1.** 溶液為									
2.1.11 The magnification power of the objective selected = 所選用的物鏡放大倍率 =									
	Panel 圖片樣版								
	4a	4b	4c	4d	4e	4f	4g	4h	4i
2.1.14 (solution chosen by you)									
2.2 (20% NaCl)									
2.3 (20% NaCl followed by solution A)									
2.4 (20% NaCl followed by solution B)									
2.5 (20% NaCl followed by solution C)									

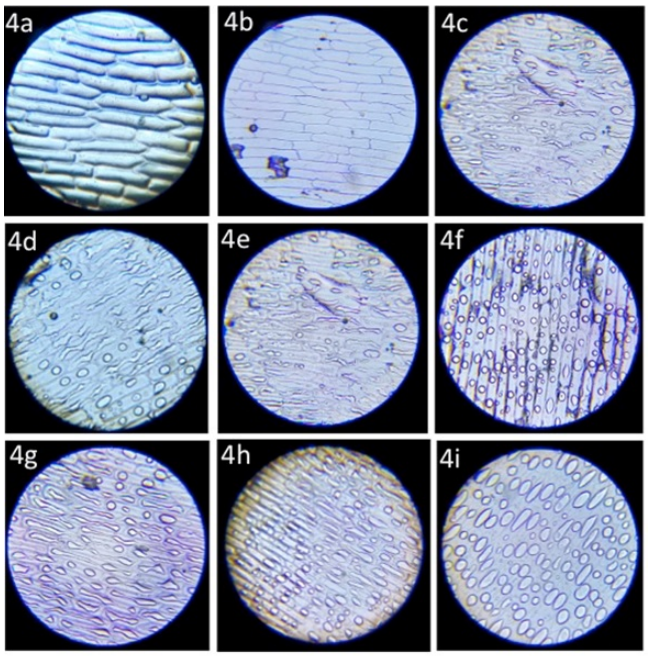


Figure 4

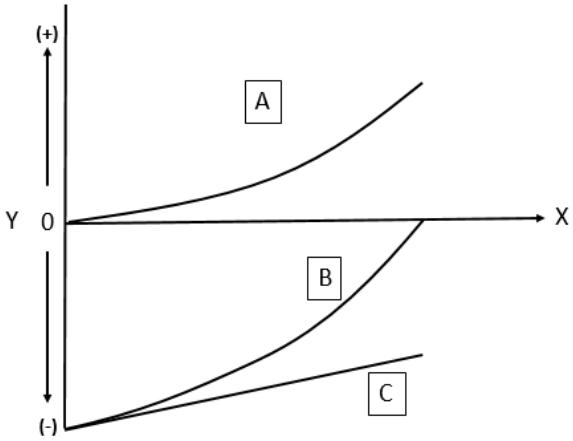
Q.2.3 Based on comparing your observations following treatments with solutions A, B and C, identify the concentration of NaCl in each of these solutions by marking a cross (X) in the appropriate cell in the table
根據你處理溶液 A, B and C 的觀察結果，判定每種溶液的 NaCl 濃度，並在表的適當空格中，以 X 標示之 5.0pt

% of NaCl	Solution A	Solution B	Solution C
0			
5			
15			

Part 2. Answer the following: 回答下列問題

The following graph shows how water potential (ψ), pressure potential (ψp) and solute potential (ψs) depend on the water content of a plant cell placed in pure water.

以下曲線圖顯示植物細胞置於純水中的水含量多寡 (X 軸) 所得水勢 (ψ)、壓力勢 (ψp) 和溶質勢之變化 (Y 軸)

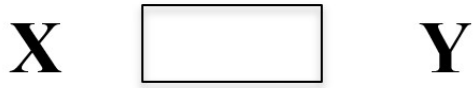


X	water content of cell 細胞含水量
Y	Pressure (MPa)

Q.2.4 What are the correct labels for the graph? Identify the potential represented by the curves A, B and C by making a 'X' in the appropriate cell in the table. 曲線圖中的正確編碼是哪一個? 在下表適當空格中，曲線 A, B and C 分別代表哪一個，以 X 標記之 3.0pt

	A	**B**	**C**
Water potential (ψ)			
Pressure potential (ψp)			
Solute potential (ψs)			

Q.2.5 X and Y are two plant cells adjacent to each other. 1.0pt
X and Y 是兩個相鄰的植物細胞
Cell X has solute potential of -9 MPa and pressure potential of 4 MPa.
細胞 X 的溶質勢為 -9 MPa，壓力勢為 4 MPa
Cell Y has solute potential of -8 MPa and pressure potential of 5 MPa.
細胞 Y 的溶質勢為 -8 MPa，壓力勢為 5 MPa
Indicate the movement of water between the two cells by marking an $\longrightarrow$ in the proper direction between cells X and Y in the box below. Mark '=' in the box, if there is no movement of water.
在空格中，用箭號 $\longrightarrow$ 顯示兩個細胞之間的水移動方向。倘若沒有水的移動，則標示為'='



Q.2.6 The solute potential of a plant cell is -8.5 MPa and its pressure potential is 1.5 MPa. The plant cell is placed in a solution with a water potential of -7 MPa. Identify the following statements as True or False by marking a cross (X) in the appropriate cell 1.0pt
某個植物細胞的溶質勢為 -8.5 MPa，壓力勢為 1.5 MPa，此植物細胞被置於水勢為-7 MPa 的溶液中。判斷下列敘述的對或錯，並在適當的空格中，以 X 標示之。

Statement 敘述	True 對	False 錯
此植物細胞的水勢為 -7 MPa		
此植物細胞會呈現原生質離		