25th INTERNATIONAL BIOLOGY OLYMPIAD

5 – 13 July, 2014

INDONESIA

PRACTICAL TEST 1 實作測驗 1

CELL & MOLECULAR BIOLOGY

細胞與分子生物學

Total points: **64.5** 總分：**64.5**

Duration: 90 minutes 時間：90 分鐘

COUNTRY:	.....
STUDENT:	.....

Dear Participants 親愛的參賽者

- In this test, you have been given the following tasks:
  本測驗中，你有以下實驗要完成

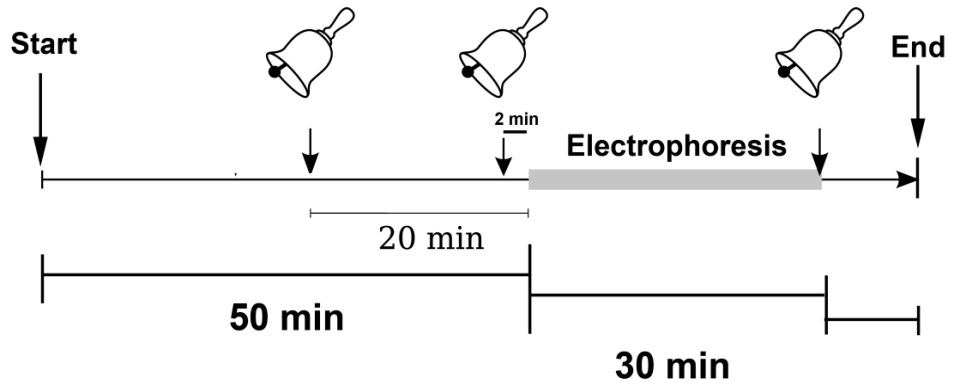
  Part A. Confirmation of plasmid samples X, Y, and Z by restriction enzyme analysis. (40 points)
  以限制酶切位分析去確認質體 X, Y 和 Z

  Part B. Cell reproduction and telomere analysis of *Paramecium*. (24.5 points)
  草履蟲細胞生殖和端粒分析

- Answer all the questions in the **Answer Sheet** provided.
  所有答案要寫在答案卷上
- The answers written in the **Question Paper** will **NOT** be evaluated.
  寫在試題卷上的答案將不給分
- Write your answers legibly in ink.
  使用原子筆清楚寫答案
- 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 immediately.**
  確認所有的材料、儀器都符合清單所列，**若發現任何項目缺少，立即舉手**
- **Use the gloves provided for the experiment**
  實驗時使用提供的手套
- Stop answering and put down your pen **immediately** when the final bell rings.
  測驗結束鈴響時，**立刻**放下筆停止做答
- At the end of the test, place the Answer Sheet and Question Paper in the envelope provided. Our Assistants will collect the envelope from you.
  測驗結束時，將答案卷和試題卷放進提供的信封套中，助理會來收取信封套
- **Note!** All the DNA electrophoresis instruments will be turned on simultaneously by the assistants **50 minutes after the start of the test.** Make sure that you have placed your samples in the agarose gel in accordance to the instructions on the Question paper before the power supply is turned on. After this, you will **NOT** be allowed to run the gel. To remind you, the assistant will ring the bell three times. Bell 1 will remind you that the electrophoresis will be carried out in 20 minutes. Bell 2 is a warning that electrophoresis will begin in two

minutes' time. Bell 3 will mark the end of electrophoresis. At that time you will be asked to remove your gel and place it into the box provided for assessment.

- **注意! 考試開始後 50 分鐘，**助理將統一開啟 DNA 電泳設備，請一定要依試卷上的指示，在開啟電源之前將樣品加入電泳膠。**錯過時間者，樣品將無法進行電泳。**如圖所示，助理將以總共三次鈴聲提醒時間，第一次鈴聲提醒電泳將在 20 分鐘內啟動電源，開始電泳前 2 分鐘會有第二次鈴聲警示，電泳結束時會有第三次鈴聲通知，此時將你的膠片(含底盤)取出，放入提供的盒中。



Materials and Equipment 材料與儀器

Materials 材料	**Quantity 數量**	**Unit 單位**
Restriction endonucleases **FD *Eco*RI** (kept on ice) 限制酶 **FD EcoRI** (冰上)	1 (8 μL)	tube 管
Restriction endonucleases **FD *Hind*III** (kept on ice) 限制酶 **FD HindIIII** (冰上)	1 (8 μL)	tube 管
10X Restriction buffer solution (labeled **FD Buffer**) kept on ice 10X(倍)限制酶反應溶液(標示 **FD Buffe**，在冰上)	1 (8 μL)	tube 管
Plasmids 1, 2 and 3 kept on ice **質體 1, 2 and 3** (冰上)	3 (5 μL)	tubes 管
Sterile water (labelled **Deion**) in zipper bag 無菌水(標示 **Deion** 小管，在冰上夾鏈袋內)	1 (100 μL)	tube 管
DNA staining solution, labeled **Gel Red** (in black tube) in zipper bag DNA 注膠染劑(標示 **Gel Red** 的黑色小管，在冰上夾鏈袋內)	1 (200 μL)	tube 管
DNA ladder stock solution, labeled **1 Kb Ladder**, kept on ice 尺標 DNA (標示 **1 Kb Ladder** 小管，在冰上)	1 (20 μL)	tube 管
Ready-made agarose gel 做好的電泳膠片	1	piece 片
Running buffer (TAE) 電泳溶液 (TAE)	1 (300 mL)	bottle 瓶

Equipment 儀器	Quantity 數量	Unit 單位
DNA electrophoresis gel tank per person DNA 電泳槽(每人一組)	1	set 組
Power supply for four persons 電源供應器(四人共用一個)	1	unit 個
Micropipettes and tips in boxes (p10, p200) p10, p200 微量分注器及吸管尖	2	sets 組
Stopwatch 計時器	1	piece 個
Tube rack 管架	1	piece 個
Sterile microtubes (in zipper bag) 無菌微量離心管(夾鏈袋內)	6	pieces 個
Plastic box 塑膠盒	1	piece 個
Floating rack styrofoam 保麗龍浮架	1	piece 個
Minicentrifuge 小離心機	1	set 臺
Sticker 標示貼片	1	sheet 張
Marker pen 奇異筆	1	piece 支
Tissue paper 面紙	1	box 盒
Gloves 手套	1	Pair 雙

Note : Use given reagents properly! No additional reagents will be provided.

注意：不提供額外材料，請小心使用

Part A (40 points)

Identification of Plasmids by Restriction Enzyme Analysis

以限制酶切位分析去辨識質體 (40 分)

Introduction 背景介紹

A scientist was surprised to find unlabelled tubes in the freezer. These tubes, containing Plasmids X, Y and Z, were arbitrarily labelled Plasmid 1, Plasmid 2 and Plasmid 3. These plasmids are indistinguishable by DNA electrophoresis because they are all 3750 bp long. Since the three plasmids differ in their restriction pattern when using *Eco*RI and/or *Hind*III (Figure 1), you will conduct restriction enzyme analysis to correctly identify the three plasmids.

一科學家在冰櫃中發現 3 個未標示的管子，這些管子分別裝有質體 X, Y,和 Z，但不知是哪一管，將此 3 個管子暫時標示為 plasmid 1、plasmid 2、plasmid 3。這些質體的大小都是 3750 bp，但以限制酶 *Eco*RI 及/或 *Hind*III 處理後之片段數目及長度不同(如圖 1)，你將進行限制酶切位分析去辨認這些質體各為何者。

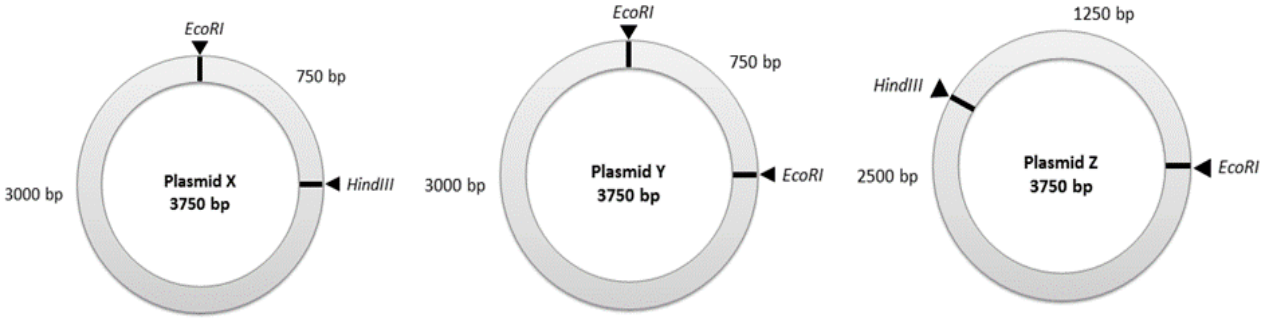


Figure 1. Restriction maps of Plasmids X, Y, Z.

圖 1. 質體 X, Y, Z 的限制酶切位圖

Table I in the **Answer Sheet** shows the experimental design you will perform. The experiment is divided into Series 1 and Series 2, carried out simultaneously. In each series, all three plasmids should be cut with the same restriction enzyme(s) The final concentration of the restriction buffer should be 1X

在答案卷的表 1 中寫出你規劃的實驗，實驗分為 Series 1 和 Series 2，同時進行。在同一 series 中，三種質體都以同樣一種（或同時二種）限制酶處理。

Question 1.1. Determine how these restriction enzyme(s) can be used to differentiate between the plasmids. Complete Table I on the **Answer Sheet to indicate your chosen protocol for each series**! (8 points). You must choose one or two enzymes for each series that are most informative based on Figure 1. If the enzyme is used in the reaction, add 1 µL enzyme.

根據圖 1 的訊息，先決定使用哪一種酵素(或同時二種酵素)處理時，可以用來區分這三種質體。在**答案卷上的表 1** 上完成你的實驗設計，將各酵素及溶液的使用量填入 (8 分)。在各 series 中，你必須使用一種(或同時二種酵素)；使用某酵素時，用量為 1 µL，填入 1。不用則填 0。

Question 1.2. Complete the figure on your **Answer Sheet** to show the expected molecular weight(s) of the completely digested plasmid for Plasmids X, Y and Z that you would observe using each of your two chosen Series. Indicate the restriction enzyme(s) used for Series 1 and Series 2 of your experimental design. (6 points – 1 points each lane).

請先勾選你在 Series 1 和 Series 2 中使用的限制酶。根據你決定的二種 series 的實驗設計，若各質體 (X, Y 和 Z) 的限制酶處理皆完全作用時，推測應該得到的片段大小，按尺標位置，將預期觀測到的片段畫在答案卷的圖上。(每一樣品 1 分，本題共 6 分)

Plasmid restriction and DNA electrophoresis protocol

質體限制酶處理及 DNA 電泳操作

Note: Electrophoresis will start 50 minutes after the test begins. After this, you will NOT be allowed to run the gel.
Note: Spin down all reagents in microtubes before pipetting (Be sure to balance the minicentrifuge by placing the microtubes opposite each other, even if there is only one tube to spin).

注意：電泳將在測驗開始後50分鐘時進行，過時則無法進行電泳。
注意：吸取各溶液前，先將管內溶液短暫離心至管底部，離心時確實將離心管放在相對位置，以維持平衡，即使只有一管離心時，也要放置另一管。

1. Label the microtubes S1 to S6, and prepare the reaction mixtures according to Table I in the Answer Sheet. Spin down the mixture by placing the microtubes in the minicentrifuge for 10-20 seconds.
將 6 個微量離心管分別標示 S1~S6，並依答案卷的表 1 規劃去配製反應混合溶液，配好後短暫離心混合溶液至管底部

2. Place the microtubes into the floating rack labelled with your bench number and incubate for at least 10 minutes at 37ºC in the water bath, located at the end of your aisle in the direction of the arrow.
將完成混合之各反應小管放在已標示你的實驗桌號碼的保麗龍浮架中，在 37ºC 水浴中至少反應 10 分鐘，水浴槽在箭頭標示方向的走道端

3. Add **ONLY 2 µL** of DNA staining solution into microtube labelled 1 Kb Ladder. Spin down the solution for 10 seconds.
在尺標 DNA 管中(標示 **1 Kb Ladder**)加入 **2 µL** 的 DNA 注膠染劑，混合，短暫離心

4. After incubation, raise your bench number and your tubes will be returned to you. Add **ONLY 1 µL** of DNA staining solution into each of these tubes, mix well by pipetting and spin down any residual liquid.

水浴反應時間到達時，舉起你的實驗桌號碼牌，有人會將反應管送回給你，在每一管中分別加入 **1 µL** 的 DNA 注膠染劑，以微量分注器混合後，短暫離心

5. Open the gel agarose package and place the gel with its tray in the electrophoresis chamber. Pour the entire TAE buffer from the bottle into the tank.

Note: Black plug is cathode (-) and red plug is anode (+).

打開電泳膠片包裝，將膠片(含底盤)放入電泳槽中，將電泳溶液瓶中的 TAE 溶液全部倒入電泳槽內。**注意：黑色接頭是 (-) 極，紅色接頭是 (+) 極**

6. Load 10 µl of samples S1 to S6 and molecular weight marker solution into the agarose gel as shown in **Figure 2**.

按照圖 2 的順序，將 10 µl 的 S1 ~ S6 及尺標 DNA 分別加入電泳膠孔

Figure 2 圖 2

7. Close the lid of the gel chamber and notify the assistant by raising your hand that you are ready. The assistant will connect the cables to the power supply.

蓋上電泳槽上蓋，舉手通知助理你已準備好，助理會連接電源線至電源供應器

During the electrophoresis continue with Part B.

進行電泳時，繼續做 Part B 的部分

8. After the electrophoresis (indicated by Bell 3 ringing), the assistants will turn off the power supply. Carefully place the gel with the tray in the plastic box provided. **Pour the running buffer from the electrophoresis chamber into the plastic box.** Close the box, label the sticker with your **Student ID** and affix it to the side of the box. Leave the box on your bench. Later the gel will be documented and the picture will be attached onto your answer sheet by the assistants.

第三次鈴聲表示電泳完成，助理會關掉電源，請小心將膠片(含底盤)自電泳槽中取出，放入塑膠盒中，**將電泳槽中的電泳溶液倒入同一塑膠盒**，蓋上盒子，在標示貼片上寫下你的**學生編號**，貼在盒子側邊，將盒子留在實驗桌上。測驗後助理會將膠片拿去照相，並將照片貼在你的答案卷上。

Part B (24.5 points)

Cell Reproduction and Telomere Analysis of *Paramecium*

草履蟲的細胞分裂與端粒分析

Two of the phenomena displayed by *Paramecium tetraurelia* are binary fission (asexual reproduction) and conjugation (exhange of genetic materials), which are affected by the abundance of nutrients in the media. Figure 3 shows microscopic observations of two *P. tetraurelia* cultures grown in rich and poor media, respectively.

草履蟲會有兩種分裂方式。其一、分裂生殖（無性生殖）；其二、接合生殖（有性生殖）。這些分裂方式會受到培養基中營養源的豐富度影響。圖 3、是在顯微鏡下觀察富源與寡源培養基生長所得之結果。

Question 2.1. (2 points) Which of the phenomena are shown by the two cultures in Figure 3A and 3B?

問題 2.1：（兩分）圖 3A 與 3B 分別觀察到何種生殖現象？

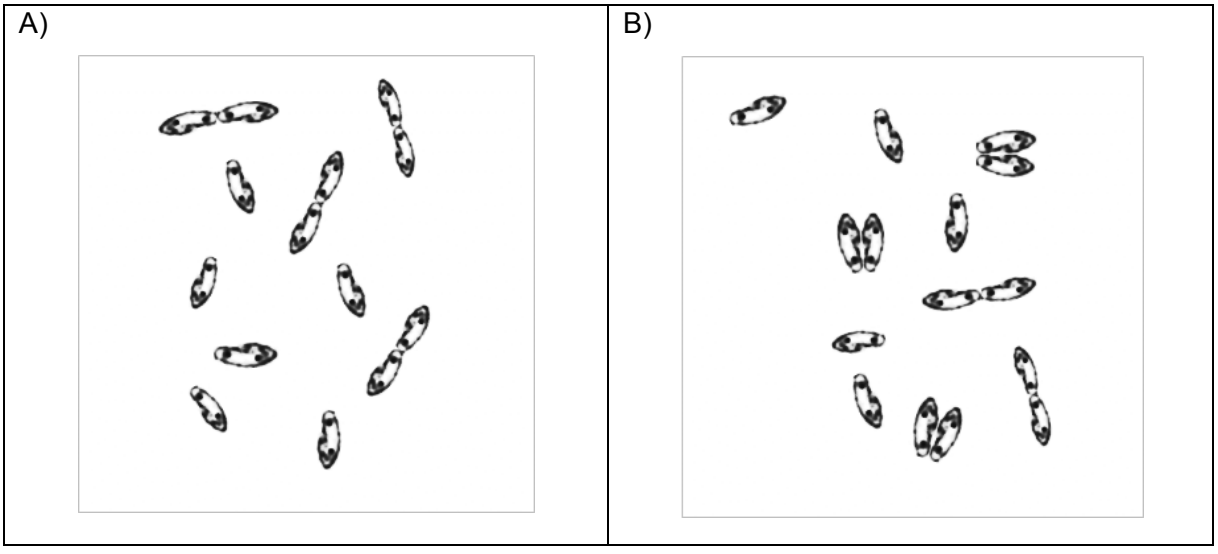


Figure 3. *P. tetraurelia* growing in rich (A) and poor (B) media.

圖 3 草履蟲分別培養於富源 (A) 與寡源 (B) 培養基中的結果。

Paramecium, which has just undergone conjugation, continues to asexually. In this instance, telomere shortening occurs for a certain number of divisions during logarithmic growth. **Table B-1** shows data for the growth of a strain of *Paramecium* in three replicates for four days. The cultures can be grown continously with no significant change in the fission rate.

剛進行完接合生殖的草履蟲，會進入無性生殖階段。此時，處於對數期生長的草履蟲，會發生端粒縮短的現象。表 B-1 所顯示的是在培養四天過程中，三次重複計算草履蟲的數目結果 。培養過程中，草履蟲的生長以及分裂速率，沒有顯著改變。

Table B-1. *P. tetraurelia* growth in the first four days.

表 B-1 草履蟲在四天中的培養結果

Day	Cell Concentration (Cell/mL)		
	A	B	C
0	1	1	1
1	8	10	16
2	80	120	128
3	640	960	1024
4	5760	7680	10240

Question 2.2. (2.5 + 8 points) Calculate the average cell concentrations and using the logarithmic value, draw the growth curve of *P. tetraurelia* from Days 0 to 4 on the graph provided in your **Answer Sheet** (Precision: Integer).

問題 2.2（2.5 + 8 分）： 分別計算第 0 ~ 4 天中平均細胞濃度，並以其對數值在答案卷所提供的空白方格圖上，畫出生長曲線。（以整數表示）

Figure 4 shows the result of Southern blot analysis of *P. tetraurelia* telomeres for a total of 30 synchronized generations of cultivation. DNA from *P. tetraurelia* was digested at the beginning of the telomere region, blotted onto a membrane and then hybridized with telomere probes. Telomeres were seen as smears, the midpoints of which can be matched with molecular weight markers to determine the average telomere length.

圖 4 表示的是培養到第 30 個同步世代草履蟲端粒的南方墨點結果。草履蟲的端粒開始部位經過酵素切割，並被轉漬到膜上，再經由端粒探針進行雜合。端粒雜合結果呈現塗暈狀，塗暈狀的中間點所對到的分子量，即代表端粒的平均長度。

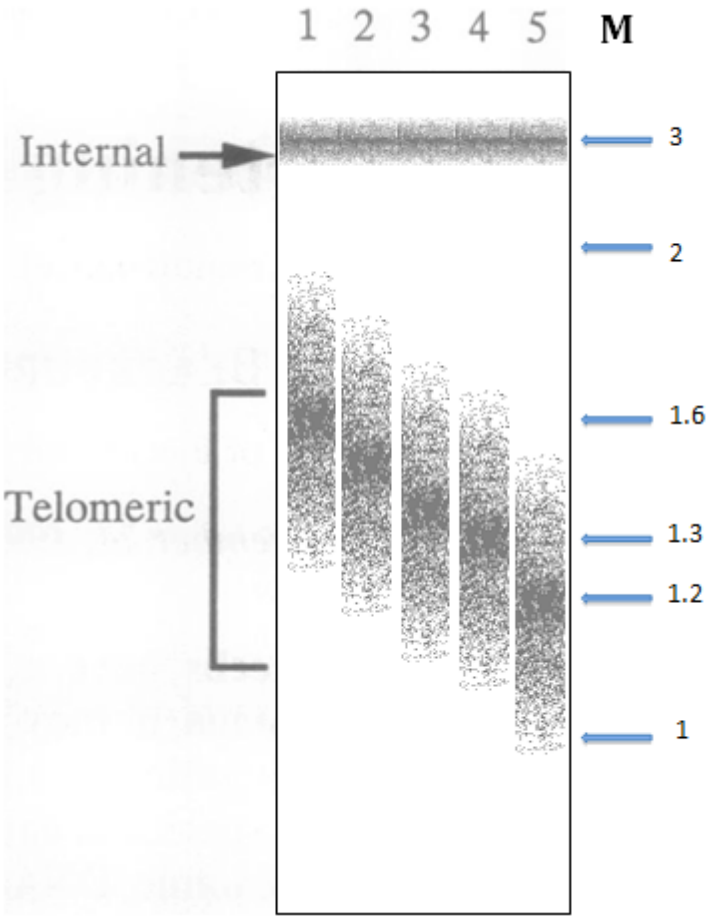


Figure 4. Southern blot of *P. tetraurelia* telomeres. Lanes 1 to 5 show telomeres after 4, 7, 17, 23, and 30 generations, respectively. DNA size markers (M) are shown next to Lane 5 (in kbp)

Internal: Internal part of the chromoeomal DNA that contains telemetric sequences)

圖 4：草履蟲端粒的南方墨點結果。第 1 ~ 5 行，分別代表第 4, 7, 17, 23 與 30 世代的結果。M 為 DNA 分子量尺標，其大小顯示于第 5 行的右側，單位為 kbp。

Internal: 染色體 DNA 樣本，其中含端粒序列

Question 2.3. (12 points) Indicate in the answer sheet with a tick (√) if the following statements are **true or false.**

問題 2.3（12 分）：關於以下敘述，請在答案卷上的 **正確** 或 **錯誤** 欄位處以打勾 (√) 標示正確判斷 。

Question 2.3. (12 points)

No.	Statement 敘述
a	The media can support the growth of *P. tetraurelia* to more than 10^4 cells/mL. 培養基能提供草履蟲生長到大於 10^4 cells/mL
b	The Telomere probe used for Southern blot analysis is not specific for telomeric sequences. 用於南方墨點法的端粒探針，對於端粒序列的專一性不足
c	Based on the Southern blot, telomeres of the cells from the same generation have the same length. 根據南方墨點法的結果，同一世代的端粒具有相同的長度
d	Paramecium can grow from 10^5 to 10^7 cells in less than three days, if enough medium is provided. 如果營養源供應充足，草履蟲能在三天內從 10^5 長到 10^7
e	Based on telomere data from lane 4 and 5, telomeres shorten by 20-25 base pairs per generation. 根據第 4 與 5 行的端粒資料，每個世代的端粒會縮短約 20-25 base pairs
f	At beginning of the experiment (parental generation), telomeres were likely between 1500 and 1700 base pairs long. 在實驗開始進行前（親代），端粒長度約在 1500 - 1700 base pairs

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5 – 13 July, 2014

INDONESIA

PRACTICAL TEST 1 實作測驗 1

CELL & MOLECULAR BIOLOGY 細胞與分子生物學

ANSWER SHEET 答案卷

Total points: **64.5** 總分：**64.5**

Duration: 90 minutes 時間：90 分鐘

COUNTRY:	.....
STUDENT ID:	.....

Task (64.5 points) 實驗(64.5 分)

Plasmid Identification and Telomere Analysis

質體分析和端粒分析

- The answers have to be given either with a tick (√) or with Arabic numbers. The numbers "1" and "7" can look very similar in handwriting. To make sure that those two numbers can be well distinguished by the IBO staff, please write them as you normally would into the following box.
  答案必須以勾選 (√) 或 數字 的方式回答，手寫數字 "1" 和 "7" 常被混淆，為確保試務人員正確判讀你的答案，請在下列方框中寫下你習慣的 "1" 和 "7" 手寫樣式

1 =		7 =	

Part A. Confirmation of plasmid sample X, Y, and Z by restriction analysis and DNA electrophoresis. (40 points)

利用限制酶切位分析和電泳分辨質體 X, Y, 和 Z

Q 1.1. (8 points)

Table I. Design of Experiment for Plasmid Identification

表 1. 辨識質體實驗規劃，填入使用量 (8 分)

No.	Reagents	Series 1 (Volume in µL)			Series 2 (Volume in µL)		
		Plasmid 1	Plasmid 2	Plasmid 3	Plasmid 1	Plasmid 2	Plasmid 3
		S1	**S2**	**S3**	**S4**	**S5**	**S6**
1	**Sterile water** 無菌水						
2	**10 X Restriction buffer solution** 10X(倍)限制酶反應溶液						
3	**DNA Plasmid DNA 質體**	**1**	**1**	**1**	**1**	**1**	**1**
4	***Eco*RI***						
5	***Hin*dIII***						
	Volume total 總體積	**10**	**10**	**10**	**10**	**10**	**10**

* If the enzyme is used in the reaction, add 1 µL enzyme

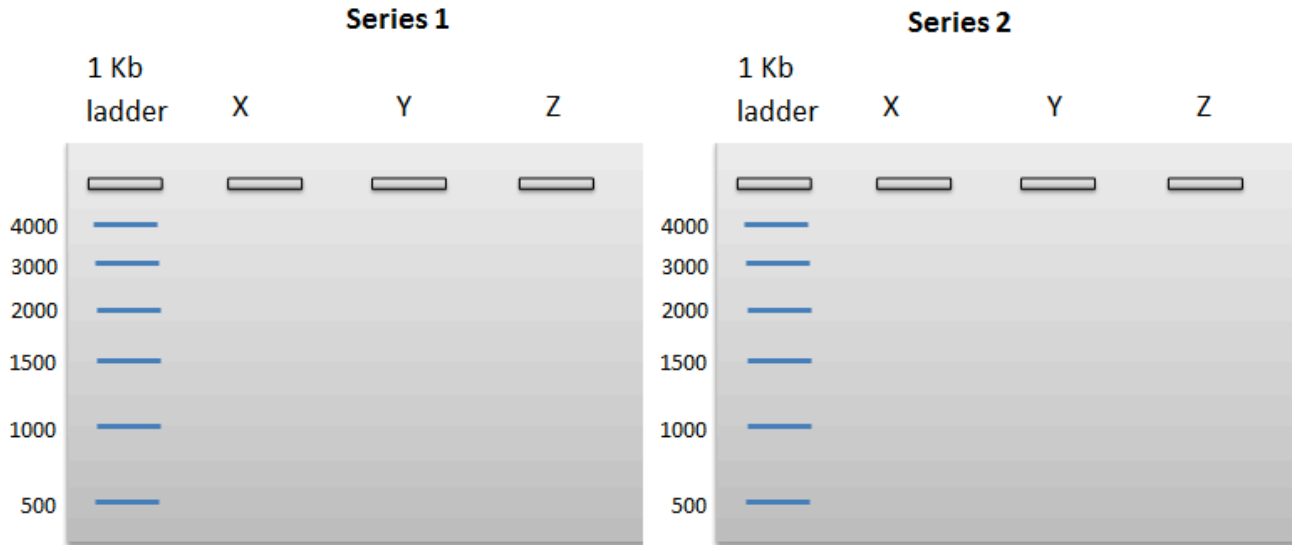
若使用此酵素，用量為 1 µL，填入 1，不用的填 0。

Q 1.2. (6 points)

Please give a tick (√) to the chosen enzyme on the table below and put minus (-) if the enzyme is not chosen.

請在下表中空白欄位勾選 **(√)** 使用的限制酶，未使用的限制酶則畫減號 **(-)**

Enzyme Used	Series 1	Series 2
*EcoR*1		
*Hind*III		



Q 1.3. Photo (26 points)

電泳結果照片(26 分)

Attach photograph of gel here

Part B. Cell reproduction and telomere analysis of *Paramaecium* (24.5 points)

草履蟲的細胞分裂（複製）與端粒分析

Q 2.1. (2 points).

Please give a tick (√) for the presence and minus (-) for the absence of the phenomenon

有觀察到請打勾 (√)，沒有觀察到則以減號 (-) 標示

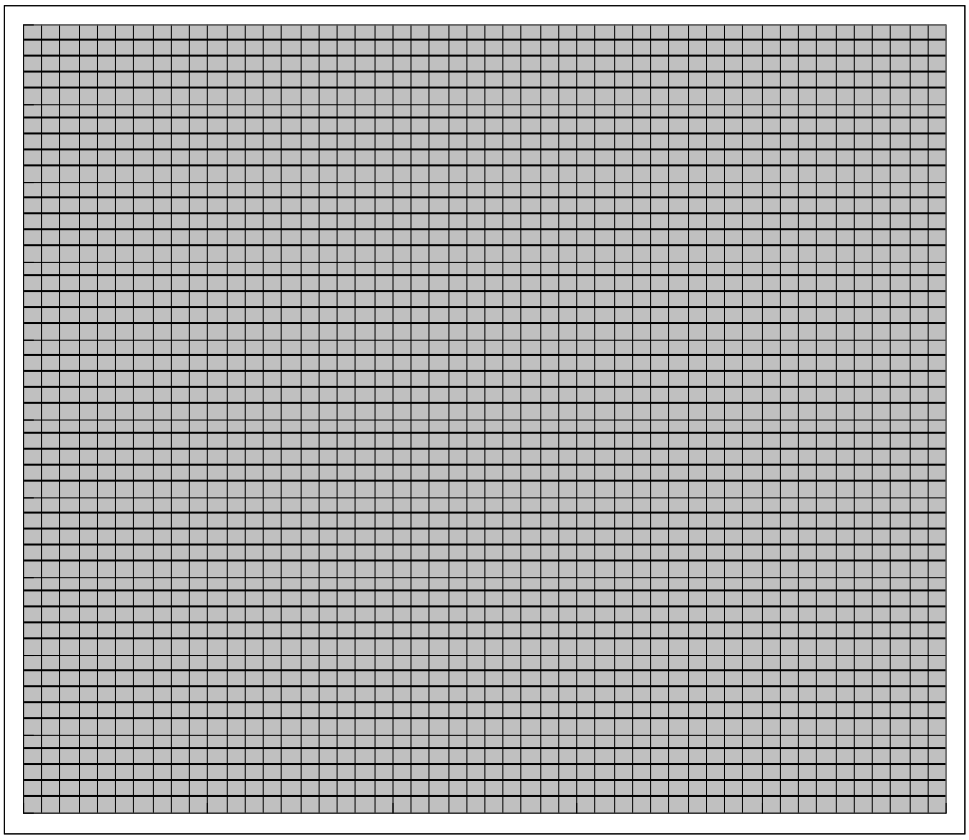
	Phenomena 現象	
Culture	**Binary Fission 分裂生殖**	**Conjugation 接合生殖**
A		
B		

Q 2.2. (**2.5 points**)

Day	**Average cell concentration (cells/mL) 平均細胞濃度 (Q2.2)**	**Log of Average cell concentration 平均細胞濃度對數值**
0		
1		
2		
3		
4		

$$N = N_o 2^n$$

Q 2.2. (8 points)



Q 2.3. (12 points)

No.	True	False
a		
b		
c		
d		
e		
f		