Country: ______________________

Student Code: ________________

23rd INTERNATIONAL BIOLOGY OLYMPIAD

8th – 15th July, 2012

SINGAPORE

PRACTICAL TEST 1

CELL & MOLECULAR BIOLOGY

細胞及分子生物學

Total points: **100**

總分：100

Duration: **90 minutes**

時間：90 分鐘

Dear Participants 親愛的參賽者

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

  在本考場中你要完成下列工作：

  Task: Gene mapping by restriction endonuclease digestion of DNA fragments

  以限制酶切割DNA片段來做基因切位圖譜

  Part A. Confirmation of insertion of human DNA in a cloning plasmid. (80 points)

  確認在一選殖質體中有插入的人類 DNA片段 (80 分)

  Part B. Determination of orientation by which the fragment is inserted. (20 points)

  決定所插入片段的排列方向 (20 分)

- Use the **<u>Answer Sheet</u>**, which is provided separately, to answer all the questions.

  所有問題作答於答案卷上。

- The answers written in the Question Paper will **<u>NOT</u>** be evaluated.

  寫在試題卷上的答案不會被評閱。

- Write your answers legibly in ink.

  用原子筆作答。

- 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**.

  檢查你得到所有的實驗材料和器材，若有短少，**立即**舉手反應。

- Stop answering and put down your pen IMMEDIATELY when the bell rings.

  鐘響時，請**立即**放下筆，停止作答。

- 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.

  測驗結束後，將答案卷和試題放入提供的信封內，實驗室助理將會來收取。

Have fun and Good Luck! ☺ 祝好運

細胞與分子生物學實作題

Materials and equipment:

實驗材料與儀器

Materials and equipment **實驗材料與儀器**	**Quantity** **數量**	**Unit** **單位**
restriction endonuclease RE1 (*Nde*I) (kept on ice) 限制酶 RE1 (*Nde*I) (置於冰上)	4 μl	tube 管
restriction endonuclease RE2 (*Eco*RI) (kept on ice) 限制酶 RE2 (*Eco*RI) (置於冰上)	4 μl	tube 管
DNA test samples in enzyme buffer (labelled T) (on ice) DNA 待測樣品溶於限制酶緩衝液中 (標示：T) (置於冰上)	10 μl x 4	tube 管
miliQ water (labelled W) miliQ 純水 (標示：W)	1	tube 管
DNA electrophoresis gel tank and power supply DNA 電泳槽和電源供應器	1	set 組
micropipettes and tips in boxes (p10, p100) 微量分注器和分注器吸管尖(p10, p100)	2	piece 件
stopwatch 計時器	1	piece 個
DNA ladder (as internal size markers, L1 for 100 bp range and L2 for 1 kbp range) (on ice) 尺標 DNA (作為樣品大小參考標準，L1 適用 於 100 bp 差異 ；L2 適用於 1 kbp 差異) (置於冰上)	2	tube 管
DNA loading dye (blue in colour) DNA 加注染料(藍色)	1	tube 管
pre-cast gel in holder (already placed in running buffer) 預先已做好的電泳膠 (已放在電泳緩衝液 running buffer 中)	1	piece 片
large petri dish (for placing the gel for imaging purposes) 大培養皿 (裝盛電泳膠片送去照相時使用)	1	piece 個

card with your country code (in a clip holder): for signalling for assistance 標記有你的國家代號的卡片：招呼協助時使用	1	piece 個
floating rack (labelled with your country code) 圓形浮水離心管座 (標記有你的國家代號)	1	piece 個
micro-centrifuge 微量離心管	1	set 組
water-bath 37 ºC (there is one assigned for your usage) 37 ºC 水浴槽(使用指定的一個)	1	set 個
gel doc (there is one assigned for your usage) 膠片照相裝置 (使用指定的一個)	1	set 個

Task (100 points)

試題 (100 分)

Gene mapping by restriction endonuclease digestion of DNA fragments

以限制酶切割 DNA 片段後推定基因切位圖

Introduction

Genetic mapping is routinely used in analysing the order and the identities of DNA fragments. This technique is based on the unique profiles of DNA fragments generated after DNA digests with specific combination of restriction endonucleases (RE) and revealed by DNA gel electrophoresis. It is extremely powerful for gene cloning, studying gene function and regulation, for finding candidate genes for diseases and their diagnosis and also as a forensic tool.

背景介紹

基因的限制酶切位圖常被使用於分析 DNA 片段的順序和內容。一個 DNA 片段經限制酶切割，並透過電泳分離後會顯示此 DNA 片段特有的模式。此技術在選殖基因、研究基因功能和調控、尋找疾病相關基因和疾病診斷，及法醫分析上均為有用的工具。

Part A. Confirmation of insertion of human DNA in a cloning plasmid. (**80 points**)

Using this technique, you are now tasked to confirm that a fragment of human DNA “X” (approximate size: 760 base pairs) has been inserted into a cloning plasmid or vector “V” (circular and approximate size: 2570 base pairs). You are required to design and carry out DNA digests by incubating DNA “T” with the restriction endonucleases by following the general protocol of incubation and electrophoresis given (details described below). After the gel electrophoresis, your results will be revealed by DNA staining (this will be performed by lab technicians), analysed and data interpreted.

A部分. 確認在一選殖質體上含有插入的人類DNA片段 **(80 分)**

你要利用限制酶切位分析法去確認一個人類 DNA 片段 “X” (長度大約是 760 base pairs)是否已被插入到一個選殖質體 “V” 內 (V 是環狀 DNA，長度大約是 2570 base pairs)。你要先設計排定一個 DNA 的限制酶切割實驗去分析樣品 DNA “T”，並且遵循一般限制酶使用和電泳操作程序(詳述於後)去操作實驗。電泳完成後，電泳膠片將會有實驗室助理協助染色，請對實驗結果進行分析並作出解釋。

Protocol and Procedures

實驗步驟

1. Design your DNA digests (you may do a maximum of 4 tubes) in a total volume of 20 μl by using the Table in **the Answer Sheet**.

   在答案卷上的表格內排定你的DNA限制酶切割實驗，每個切割項目的體積為 20 μl，你最多可以做 4 個切割項目。

   Q1.1 **(20 points)** Record the desired amounts of reagents in your plan. One example is already given for Tube 2 in the table provided. All units are in μl.

   (20 分) 寫下你計畫的每個項目中各種成分的用量，如Tube 2 項下所示，所有的單位都是 μl。

2. Prepare the mixtures by carefully pipetting the correct amount of the reagents and gently mix them by pipetting them up and down in each tube. Label the tubes. Do not contaminate one sample with another when preparing the mixture. Use a clean pipette tip for each operation. Note: use p10 micropipette (white-coded) for pipetting reagents of less than 10 μl. [NOTE: there will be a penalty of 20 points if additional samples are requested. Please prepare the samples carefully.]

   依你的實驗設計來標示微量離心管，小心吸取正確定量的各項成分，用微量分注器輕緩混合於各反應微量離心管內。要小心避免各項處理項目間的相互污染，每次吸取時要用乾淨未用過的吸管尖。注意：使用 p10 分注器 (白色頂)吸取 10 μl 以下的量。[注意：如果你要求額外的樣品，會被扣 20 分，請小心使用你的反應樣品]

3. Spin down the mixture by placing all four tubes in the micro-centrifuge (please balance the spin by placing tubes opposite to each other). During preparation and after spinning, always keep the tubes on ice.

   用迷你離心機將 4 管反應混合液離心至離心管底部 (離心時請平衡放置離心管於相對位置)。準備反應混合液時及離心後，隨時將離心管放在冰上。

4. After all the tubes have been prepared, remove them from the ice and place them into the labelled floatation rack and incubate them for 20 minutes (stopwatch is provided) at 37 ºC in the water bath assigned to you. Make sure that you retrieve your own samples after the 20 minutes incubation time.

   當所有反應混合液都準備好後，將離心管置於標記好的浮水離心管座，放在指定的 37 ºC 水浴中反應 20 分鐘(以計時器計時)。記得 20 分鐘後要取回你的實驗樣品。

5. During this 20 minute incubation duration, answer the following questions **in the Answer Sheet**:

   利用 20 分鐘的反應時間回答答案卷上的問題。

Q1.2 **(2 points × 5 = 10 points)** Indicate true statement(s) with a tick (✓) and false statement(s) with a cross (✗).

(2 分 × 5 = 10 分) 對下列各項敘述，正確的請打勾(✓)，錯誤的請打叉(✗)。

a. Each RE cuts DNA at a specific sequence.

每一種限制酶在特定的序列上切斷 DNA

b. Each RE cuts DNA only at the 3' and 5'ends.

每一種限制酶只在 DNA 的 3' 和 5'末端切 DNA.

c. RE are most effective in digesting DNA at 4℃.

限制酶切 DNA 的作用在 4℃時最有效

d. RE can be kept at room temperature for months.

限制酶可以在室溫中保存數月不變質。

e. Unlike exonucleases, RE only cuts DNA internally.

不同於外切酶，限制酶只能作用在 DNA 的內部序列。

Q1.3 **(2 points × 5 = 10 points)** Which of the following principles is true of separating DNA by gel electrophoresis? Indicate true statement(s) with a tick (✓) and false statement(s) with a cross (×).

(2 分 × 5 = 10 分) 對下列有關電泳分離DNA原理的敘述，正確的請打勾(✓)，錯誤的請打叉(×)。

a. DNA fragments are overall positively charged.
整體而言，DNA 片段是帶正電荷。

b. The smaller DNA fragments move faster across the gel under the electric current.
較小的 DNA 片段在電流下通過膠片的速度較快。

c. The smaller DNA fragments are lesser charged than the larger fragments hence they move faster across the gel.
較小的 DNA 片段因為比較大的片段帶電荷為少，所以通過膠片較快。

d. The relative density of the gel matrix affects how long the separation takes.
膠體的相對密度影響分離 DNA 所需的時間。

e. The voltage applied to the electrophoresis is determined by how much DNA is loaded in the gel.
依照注入膠片中 DNA 的量來決定電泳時使用的電壓。

6. When the 20 minutes of DNA digests duration is up, retrieve your own tubes from the water bath.
當 20 分鐘反應時間終了時，從水浴槽取回你的樣品。

7. Add 4 μl of DNA loading-dye (blue colour). Mix them well by pipetting the mixture up and down and spin down any residual liquid using the micro-centrifuge.
在反應樣品中加入 4 μl DNA 加注染料(藍色)，以分注器混合後，離心至離心管底部。

8. Using the p100 micropipette (yellow-coded), load 15 μl of the sample mixture of DNA digests with the loading dye into the "wells" of the agarose gel provided. Make sure that you position the pipette tips carefully on top of the wells and gently deliver the mixture to the wells without spilling them. Load 15 μl of each of the Markers, L1 and L2. Add your samples according to the following scheme of lanes, starting from the left end of the gel.

使用 p100 微量分注器(黃色頂)，分別取 15 μl 的反應樣品(混合加注染料)及尺標 DNA，依照下列順序，自左至右注入膠片的樣品槽中。注意輕緩加入樣品，千萬不要流出樣品槽外。

Marker L1	Tube 1	Tube 2	Tube 3	Tube 4	Marker L2

9. Cover the gel with a lid and connect the power supply to run at 100 volts for 20 min. Please be careful and do not touch any part of the electrodes and power supply.

蓋上電泳槽蓋，連接電源，以 100 volts 進行電泳 20 分鐘，請小心不要碰觸電極和電源供應器。

10. Check regularly that the samples have entered the wells and are indeed running towards the positive electrode. If you need help from the technicians to ensure proper runs for the samples, please signal for assistance by clipping your signal card at the edge of right wall of your cubicle.
留意樣品是否向正極方向移動。如果你需要協助去確認樣品是否正確移動，請將你的協助卡夾在實驗隔間的右側壁上。

11. While waiting for the gel run, answer the following questions **<u>in the Answer Sheet:</u>**
進行電泳時，回答下列問題<u>於答案卷上</u>

Q1.4 **<u>(20 points)</u>** Consider the following scenario: A piece of linear human DNA (1 kbp) was digested by a particular enzyme RE3, resulting in 2 fragments of 650 bp and 350 bp. The same piece of 1 kbp DNA was digested with another enzyme RE4, releasing 2 fragments of 800 bp and 200 bp. And when this 1 kbp DNA was digested with RE3 and RE4 together, 3 fragments of DNA were generated, 650 bp, 200 bp and 150 bp.

<u>(20 分)</u> 假設有一段線性的人類DNA (1 kbp)，用特定限制酶RE3 切時，得到二個片段，一個 650 bp和一個 350 bp。若用特定限制酶RE4 切時，得到一個 800 bp片段和一個 200 bp 片段。若同時用RE3 和RE4 切時，得到三個片段，分別是 650 bp, 200 bp 和 150 bp

Sketch a linear map of this piece of DNA by indicating the position of RE3 and RE4 digests in the space provided. An example of such a sketch is provided below as a guide.

按照下方的例圖模式，畫出一直線限制酶切位圖，並標出 RE3 和 RE4 的切位及切出的片段大小。

12. When the 20 minutes of gel running time is up, turn off the power supply and remove the lid of the gel tank. Carefully remove the gel (still on the gel tray) and place it on the petri dish provided. Bring your gel to the gel doc that has been assigned for your usage and the technician will photograph it for you.
20 分鐘電泳結束後，關閉電源，打開電泳槽上蓋。小心取出膠片(仍然留在膠片板上)，放在培養皿上，帶至指定的膠片照相裝置處，實驗室助理會幫你將膠片照像。

13. Bring your gel and the photograph back to your cubicle and use your signal card to get assistance for an invigilator to staple it in the space provided **on the Answer Sheet**.
取回你的膠片和照片 ，回到實驗隔間，使用你的協助卡通知監考人員將照片訂在答案卷上。

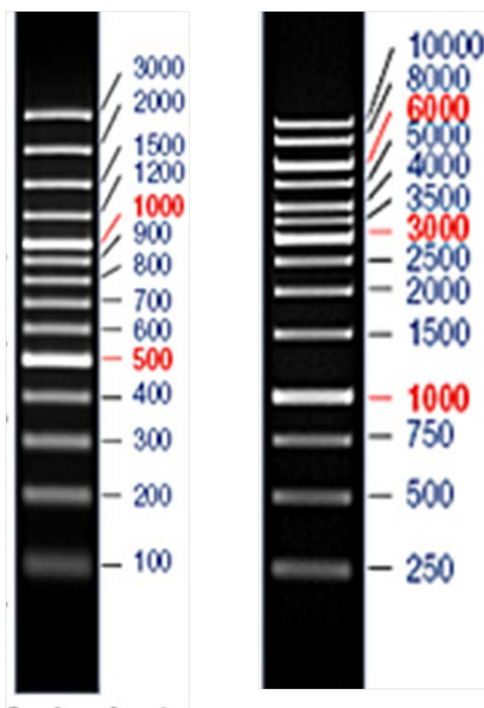
Q1.5 (10 points) Your skills in running a gel will be assessed by the quality of the gel produced.
(10 分) 你的實驗技術會由你的電泳結果來評定。

14. Based on the gel results, answer the following questions **in the Answer Sheet**:

依據你的電泳膠結果，回答下列問題於答案卷上。

Q1.6 **(1 point × 5 = 5 points)** Using the DNA ladder markers (in basepairs) provided below as the reference, estimate the sizes of the fragments/bands. You may draw a line across the band of your query and the size marker to do the estimation. How many fragment(s) of DNA were generated by RE1 and RE2? And what is/are the estimated size(s)? Answer using numerals.

(1 分 × 5 = 5 分) 根據下方尺標DNA分離模式的標準圖(以basepair顯示)，估計你的電泳結果中各片段的大小，你可以在你的膠片照片上的片段和尺標DNA間畫線來幫助估計。RE1 和 RE2 分別切出幾個片段？估計片段的大小分別是多少？



L1: 100 bp DNA Ladder L2: 1 kb DNA Ladder

Q1.7 **(1 point)** What is the estimated size of the test DNA sample (T)? Answer using numerals.

(1 分) 估計DNA樣品 (T) 的大小是多少？

Q1.8 **(1 point)** Based on your results, is the test DNA sample (T) larger, smaller or the same size as the empty vector? Indicate your answer with a tick (✓) in the correct box.

(1 分) 根據你的實驗結果，DNA樣品 (T) 與空的載體(vector)相比，是較大？較小？還是一樣大？在正確的答案空格內打勾 (✓)。

Q1.9 **(1 point)** Does the test DNA sample (T) contain any insert? Indicate yes with a tick (✓) and no with a cross (×).

(1 分) DNA樣品 (T) 是否攜帶插入的DNA片段？若有攜帶打勾 (✓) ，沒有則打叉(×)。

Q1.10 (2 points) Uncut DNA appears to move faster than any of the samples digested with RE2. Why? Indicate your answer with a tick (✓) in the correct box.

(2 分) 沒有切的DNA質體在電泳時移動的比RE2 切出的片段都要快，原因是甚麼？在正確的答案空格內打勾 (✓)。

a. The smaller fragment size of uncut DNA is due to DNA degradation.

因為沒有切的 DNA 被分解成為較小的片段。

b. The uncut DNA is more compact and therefore moves faster through the gel.

因為沒有切的 DNA 構形較緊密，所以在膠片上移動較快。

c. RE2 still binds to the DNA and therefore slows down their movement through gel.

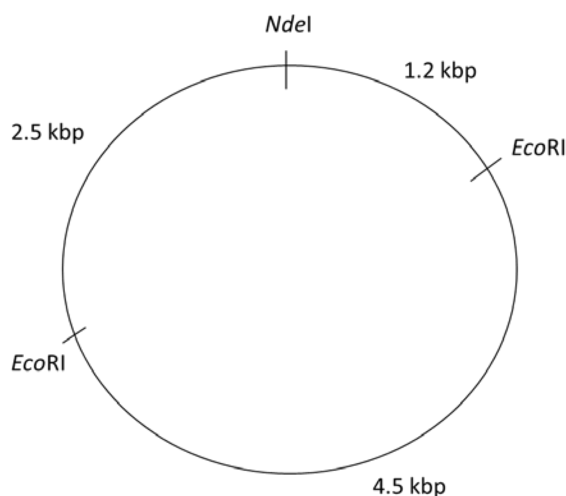
因為 RE2 依然附在 DNA 片段上，所以被 RE2 切的片段移動較慢。

Part B. Determination of orientation by which fragment was inserted. (**20 points**)

B部分. 請決定插入DNA片段的排列方向。**(20 分)**

Q1.11 **(20 points)** Construct possible restriction map(s) for the DNA "T" by indicating the relative position of RE1 and RE2 and the distance between them **in the Answer Sheet**. An example of such a map is provided below as a guide.

(20 分) 按照下方的例圖模式，在答案卷上畫出DNA "T" 所有可能的限制酶切位圖，標出RE1 和 RE2 的相關切位，以及各切位間的距離。



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