IBO 2018, Tehran, Iran

Practical Exam "Biochemistry & Molecular Biology"

**Student Code:**

29th International Biology Olympiad

15-22 July 2018, Tehran, Iran

# IBO 2018
## Tehran, Iran

**29th International Biology Olympiad**
**July 15-22, 2018**

Practical Exam
# Biochemistry & Molecular Biology

Total Points: **100**
Duration: **90 minutes**

### General information

一般資訊

-Protocol instructions and Answer sheet will be provided in your cabins within an envelope.

-實作操作說明與答案紙放在你的實驗操作區上的信封裏。

- Total points: 100
- 總分：100

- Exam time: 90 minutes
- 考試時間：90分鐘

- Please write your student code into the box on the upper left side of the title page.
- 請將您的學生代碼寫入標題頁左上角的框中。

-Write all answers on the **answer sheet**, which is provided as separate sheet. Points will not be given to answers written on the question paper**.**
- 在答案紙上寫下所有答案，這是單獨的表格。答案如寫在試題卷上將不予計分。

- In order to show a flag, place it in the **flag stand** (=small tube) located on the left wall of your desk.
- 為了顯示小旗，請將其放在桌子左側上的旗架 （=小管）中。

-Please ensure that all the materials and equipments are present in your cabin. If anything is missing, you must report it **within five minutes** after start whistle by showing your yellow flag in the flag. Report of item(s) missing after the five minutes will not be considered.
- 請確保您的實驗操作區內的所有材料和設備。如果缺少任何東西，您必須在哨聲開始響後五分鐘內用黃旗來提出報告。五分鐘後未報告所缺少的材料或器材項目將不予補全。

-In case of emergency, put your yellow flag in the flag stand.
- 如遇緊急情況，請將您的黃旗放在旗架上。

-Additional materials will not be provided in any case of material loss during the experiments.
- 不會補充在實驗過程中所耗損的任何材料。

-We suggest you familiarize yourself with the experiments before starting by reading the entire protocol before starting.
- 我們建議您在實驗開始之前，先熟讀實驗過程與步驟說明來熟悉實驗。

-Stop answering **immediately** when the stop whistle is blown at the end of the exam. Put the entire protocol along with the answer sheet in the envelope. Lab assistants will collect the envelopes.
- 在考試結束時停止哨聲時應立即停止作答。將整個實驗試題卷與答案紙一起放入信封中。實驗室助理將會收齊信封。

-In case you have placed your green flag in the flag stand, but picture of you gel has not been taken by the time that the stop whistle blows, stand up next to your cabin and wait until an assistant comes and takes the picture.
- 如果你已經把綠旗放在旗架上，但是當停止吹哨響時，你的凝膠圖片還沒有拍完，請站在你的實驗位置旁邊，等助理來拍照。

Good luck
祝你好運

Write the indicated number in the tables below.
在下表中寫下指示的數字。(協助閱卷者分辨手寫的1和7)

| | |
|---|---|
| **1** | |

| | |
|---|---|
| **7** | |

# PRACTICAL EXAM OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

**Materials: CHECK AND REPORT ITEMS MISSING WITHIN FIVE MINUTES AFTER WHISTLE**

材料： 請在哨音之後五分鐘內報告任何缺失的物品

**A. Biological**

1. 500 µl bacterial lysate (labeled “Bacterial lysate BL”; in box)
2. 500 µl BSA solution, 1 mg/ml (labeled “BSA”; in box)
3. 30 µl plasmid DNA, 250 ng/µl (labeled “plasmid DNA”; in box)
4. 10 µl DNase (labeled “DNase, 0.015 Units/µl”) **on ice**
5. 20 µl DNA size marker (labeled “DNA size marker”; in box)

**A.**生物性材料

1.500 µl 細菌裂解液（標記為“Bacterial lysate BL”;在盒子中）
2.500 µl BSA溶液，1 mg / ml（標記為“BSA”;在盒子中）
3.30 µl 質體DNA，250 ng /µl（標記為“plasmid DNA”;在盒子中）
4.冰上的10 µl的DNase（標記為“DNase, 0.015 Units/µl”）
5.20 µl的DNA大小標記（標記為“DNA size marker”;在盒子中）

**B. Non-Biological**

1. Waste bucket (labeled “Waste bucket”)
2. 1 ml Lysis Buffer (labeled “Lysis buffer LB”; in box)
3. 2 ml Buffer A (distributed in two tubes, each labeled “Buffer A”; in box)
4. 1 ml Buffer B (labeled “Buffer B”; in box)
5. 3 ml phosphate buffered saline (distributed in two tubes, each labeled “PBS”; in box)
6. 8 µl DNase reaction buffer (labeled “DNase buffer”) (**on ice**)
7. 50 µl gel loading dye (labeled “Loading dye”; in box)
8. 26 X 1.5 ml tubes (in box)
9. 4.2 ml Bradford reagent (in 15 ml tube) (labeled “Bradford reagent”)
10. Affinity chromatography column (labeled “Column”; placed on column holder rack)
11. Micropipette Stopper Tip (labeled “MST")
12. Column holder rack (labeled “Column holder rack”)
13. Tube rack for fraction collection (labeled “Tube rack”)
14. 2-20 µl Micropipette
15. 20-200 µl Micropipette
16. 100-1000 µl Micropipette
17. Yellow tips for 2-20 µl and 20-200 µl Micropipettes
18. Blue tips for 100-1000 µl Micropipette
19. 96 well plate (with student’s name)
20. Aluminum foil
21. Agarose gel electrophoresis system with incorporated power supply

22. Agarose gel containing DNA-binding stain (already placed in electrophoresis system)
23. Disposable gloves
24. Goggles
25. Water proof pen marker
26. Three flags, colored red, green, and yellow
27. Flag stand (=small tube) located on the left wall of your desk
28. Your name tag (placed on shelf)

**B.**非生物性材料

1. 垃圾桶（標有“Waste bucket”）
2. 1 ml 裂解緩衝液（標記為“Lysis buffer LB”;在盒子中）
3. 2 ml 緩衝液 A（分配在兩個管中，每個管標記為“Buffer A”;在盒子中）
4. 1 ml 緩衝液 B（標記為“Buffer B”;在盒子中）
5. 3 ml 磷酸鹽緩衝鹽水液（分配在兩個管中，每個標記為“PBS”;在盒子中）
6. 8 µl DNase反應緩衝液（標記為“DNase buffer”）（ 在冰上 ）
7. 50 µl 凝膠上樣染料（標記為“Loading dye”;在盒子中）
8. 26 X 1.5 ml管（盒裝）
9. 4.2 ml Bradford試劑（在15 ml 管中）（標記為“Bradford reagent”）
10. 親和層析管柱（標記為“Column”; 置於柱架上）
11. 微量吸管止動尖（標記為“MST”）
12. 柱架（標記為“Column holder rack”）
13. 用於層析管柱收集液的管架（標記為“Tube rack”）
14. 2-20 µl微量吸管尖
15. 20-200 µl微量吸管尖
16. 100-1000 µl微量吸管尖
17. 用於2-20 µl和20-200 µl微量吸管的黃色吸管尖
18. 100-1000 µl微量吸管的藍色吸管尖
19. 96孔盤（註記學生姓名）
20. 鋁箔紙
21. 附帶有電源供應的瓊脂凝膠電泳系統
22. 含有DNA結合染色劑的瓊脂凝膠（已置於電泳系統中）
23. 一次性手套
24. 護目鏡
25. 防水簽字筆
26. 三面旗幟，紅色，綠色和黃色
27. 位於桌子左牆的旗架（=小管）
28. 你的名牌（放在架子上）

**We suggest that you familiarize yourself with the experiment by reading the entire text below before starting the experiment**
建議你在實驗前先熟讀以下內文

**Introduction**
In this experiment, you will test the ability of a protein named Pep (that is positively charged under the experimental conditions) to interact with DNA. You will be supplied with the DNA to be tested, but you must purify the protein Pep from a crude bacterial lysate. The bacteria had previously been transformed with a plasmid expression vector into which the Pep encoding gene with a histidine tag had been cloned. Purification will be done by affinity chromatography. The histidine tag has affinity for and binds to nickel which is attached to the resin in the columns. After binding, the protein can be detached from the resin by changes of buffers used in the chromatography protocol. Eluted fractions will be collected in several tubes. You will determine the protein concentration in two of the fractions by the Bradford method. This is a colorimetric assay in which

attachment of Comassie brilliant blue to protein results in increased absorbance at wavelength of 595 nm. By using a standard curve derived by assay of a bovine serum albumin (BSA) protein solution of known concentration, the protein concentration of the fractions can be determined. Subsequently, you will test the ability of the Pep protein in one of the fractions to interact with DNA by performing a gel retardation assay. In this assay, interaction of DNA with protein retards the migration of the DNA on agarose gels during electrophoresis.

此實驗你將測試名為 Pep 的蛋白質 (此蛋白質於此實驗條件下帶正電荷)與DNA的交互作用。大會會提供你DNA，但你必須由細菌裂解液中純化出 Pep 蛋白質。

此細菌已先以質體表現載體轉型帶入可轉譯出 Pep 蛋白質的編碼基因(以 histidine tag標記)，並以親和性管柱層析來純化Pep 蛋白質。histidine tag 帶有鎳，可附著於親和性管柱層析管中的樹脂。

Pep 蛋白質經層析附著後，可以藉由通入不同的緩衝液來分離出Pep 蛋白質。以數支管子收集分餾液，再以Bradford method檢測其中兩管的Pep 蛋白質濃度。

此呈色測定蛋白質濃度的方法，是藉 Bradford reagent 中的 Comassie brilliant blue附著於蛋白質，以增加在波長 595 nm 的吸光度。

利用已知濃度的牛血清蛋白 (BSA) 作成標準濃度曲線，以插入法算出分離出的Pep 蛋白濃度。接著以凝膠阻滯電泳分析方法，測試其中一管Pep 蛋白質是否與DNA相互作用。此凝膠阻滯電泳分析法的原理是蛋白質如果與DNA相互作用，將於跑膠電泳分析時，DNA的移動會被阻滯而變慢.。

## Protocol
## 實驗操作流程

**A. Purification of Pep protein from bacterial lysate by affinity chromatography**
以親和性管柱層析從細菌裂解液中純化出 Pep 蛋白質

1. Take note of the chromatography column that is already placed in the hole of the column holder rack. Also take note that it fits into the hole tightly. Avoid having to remove it from the hole during the course of the experiment. The column is sealed at the bottom and the resin in the column is covered with a small volume of ethanol. Look carefully at the contents of the column to correctly detect the border between resin and the overlying liquid.

注意親和性管柱層析管已置放於管架上，切記在**實驗過程中不要移動它**。 在管柱下層的樹脂有少量酒精覆蓋，要非常小心觀察管柱內的內含物，並正確地辨識出樹脂與覆蓋其上液體的交界處。

2. Open the red cover on top of the column and remove the micropipette stopper tip (MST) at the bottom of the column, and quickly place tube #1 in the tube rack under the column in order to collect the eluting ethanol. Collection should continue just until there is no ethanol left above the resin. Three to four drops will be collected.
打開管柱層析管上面的紅色蓋子, 並移去管柱層析管底部的微量吸管止動尖(MST)，然後快速將管 #1放置於管架上，以收集濾出的酒精。須連續收集至不再有酒精殘留在樹脂上，將可收集到3~4 滴.

3. Quickly, but gently add 500 µl lysis buffer to the column without disrupting the resin within the column in order to equilibrate the column with LB.

快速且溫和地加入 500 µl lysis buffer LB到管柱層析管中。為了以LB平衡管柱，切勿攪動管柱內的樹脂。

4. Collect the eluting drops in tube #2 just until there is no buffer left above the resin. (Approximately 500 µl will be collected.)
收集濾出液於管 #2中，直到樹脂上不再有殘留的緩衝液 (大約可收集到500 µl)。

5. Quickly, but gently add 500 µl bacterial lysate onto the column and start collecting drops in tube #3. Be sure that all the BL has entered the column. (Approximately 500 µl will be collected.)
快速且溫和地加入 500 µl bacterial lysate (細菌裂解液) 到管柱層析管中, 並開始收集濾出液於管 #3 中，務必確認全部的細菌裂解液都進入管柱層析管中 (大約可收集到500 µl)。

6. Quickly, but gently add 500 µl wash buffer A and start collecting drops in tube #4. As the volume of the buffer above the resin decreases to about 100-200 µl, add 300 µl additional buffer A and continue collection of drops in the same tube #4. Continue with addition of 300 µl three more times (i.e. column should be washed with a total volume of 1.7 ml buffer A). Collection can be continued in tube #5. (Approximately 1.7 ml will be collected in total in tubes # 4 and 5.)

快速且溫和地加 500 µl wash buffer A 到管柱層析管中，並開始收集濾出液於管 #4 中。

當樹脂上的 wash buffer A減少至約100-200 µl時，再加 300 µl wash buffer A 於管柱中，並以管 #4繼續收集濾出液。

重複此步驟3 次 (管柱必須總共以至少 1.7 ml buffer A 沖洗)，接續以管 #4收集濾出液(管#4與管#5大約共可收集到1.7 ml)。

7. Quickly, but gently add 500 µl buffer B to start detachment of Pep molecules bound to the resin in the column. Start collecting drops in tubes #6, 7, and 8. Three drops should be collected in each tube. As the volume of the buffer above the resin decreases to about 200 µl, add 200 µl additional buffer B and continue collection of the eluent just until three drops have been collected in tube #8.

快速且溫和地加 500 µl buffer B於樹脂上，以分離附著於樹脂的Pep 蛋白質。

濾出液以管 # 6、管 #7、管 #8 收集, 每支管子收集3 滴。

當樹脂上的緩衝液減少至約200 µl時，再加入 200 µl buffer B，並以管 #8 繼續收集濾出液 (至少收集 3 滴)。

8. Quickly seal the bottom of the column with micropipette stopper tip (MST) by inserting the bottom of column into the wide mouth of the tip.

快速以微量吸管止動尖(MST)封住管柱底部 (以吸管止動尖的寬端插入管柱的底部)。

## B. Bradford protein assay (this task has two parts)
## 以Bradford 試劑進行蛋白質分析 (此Task分成兩部分進行)

**Part 1**
Prepare BSA (= the standard protein) dilutions as shown in Table 1:
配製BSA (標準蛋白) 的稀釋液，如表一 所示。

***Table 1** 表一*

| Tube # | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|
| BSA (1 mg/ml) | 0 µl | 20 µl | 40 µl | 60 µl | 80 µl | 100 µl | 120 µl |
| PBS | 200 µl | 180 µl | 160 µl | 140 µl | 120 µl | 100 µl | 80 µl |

2. Prepare dilutions of tubes #7 and #8 in tubes #16 and #17 as shown in Table 2:
配製管#7及管#8之稀釋液於管#16及管#17，如表二：

***Table 2***

| Tube # | 16 | 17 |
|---|---|---|
| Buffer B eluent fractions | 管7取40 µl | 管8取40 µl |
| PBS | 20 µl | 20 µl |

3. Mix contents of each of the tubes (tube 9 through tube 17).
分別混合管# 9 至管#17的內含物。

4. Each sample will be assayed in duplicate. For this purpose, add 10 µl of tubes #9-15 into wells of B1-B7, and then again to wells of D1-D7 of your 96 well plate. Subsequently, add 10 µl of tubes #16 into B9 and D9, and 10 µl of tube #17 to wells B11 and D11. Add 190 µl of the Bradford reagent into each of the wells to which samples had been added. Mix gently with micropipette tip and take care not to create bubbles. Bubbles would interfere with absorbance measurements.

每個樣品將重複分析兩次，因此，分別取管#9至管#15的內含物，各10 µl放入編號 B1-B7 的96-well 孔盤中，然後再各取10 µl放入編號 D1-D7 的well中。

接著取管 #16 的內含物，各10 µl分別放入編號B9 and D9 的well中。

再取管 #17 的內含物，各10 µl分別放入編號 B11 and D11的well中。

最後於每個well的樣品中，各加 190 µl Bradford reagent，並以微量吸管的管尖溫和地混合。

切記勿產生氣泡 (氣泡會影響吸光度的準確性)

5. Place a lid on the 96 well plate and wrap the plate with aluminium foil to prevent exposure to light. Incubation in the dark should continue for 5 minutes. (You may start Part 2 of this task during the 5 minute incubation.)

將96個well 孔盤的蓋子蓋好，並以鋁箔紙封好以防照光，並放置於黑暗中靜置5 分鐘 (你可於此時進行第二部分實驗)。

6. After completion of the 5 minute incubation, placed your red flag in your flag stand. A lab assistant will take your coded plate to a Spectrophotometer station and have

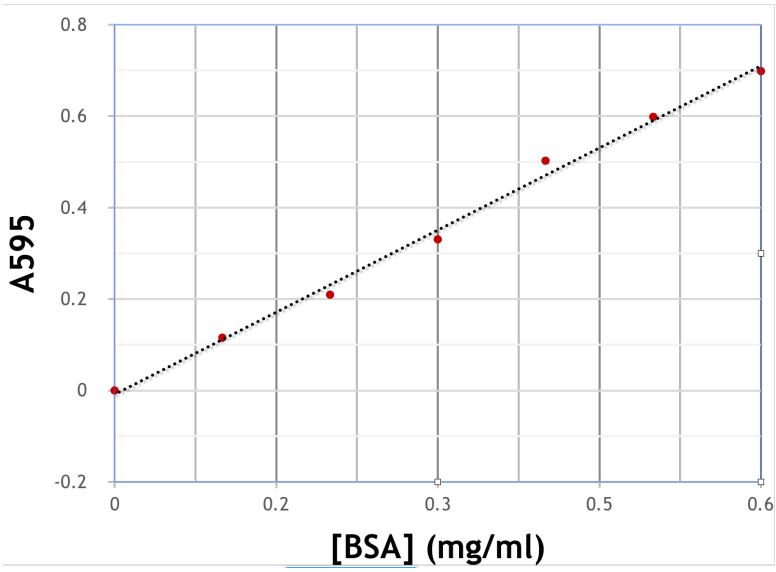
absorbance of all your wells read at wavelength of 595 nm. The absorbance readings will be used to score your performance. (45 POINTS).

當你完成以上5分鐘的實驗，請將紅旗置放於旗架上 ，助理會來取走你已註記的孔盤，去讀取 595 nm的吸光度。這些吸光度數據會用來評分 (45分)

**Part 2 NOTE: All data presented in Part 2 of the Bradford assay pertain to experiments performed earlier by an examiner.**

注意: 在**Bradford assay**的第二部分所呈現的數據，是之前已完成的結果。

1. The Bradford assay described in Part 1 was earlier performed by an examiner, and the absorbance readings were used to draw a standard curve provided below.
利用之前完成的第－部分 Bradford assay所得之吸光度，畫出以下的濃度標準曲線圖。




2. Using the standard curve and the A595 (absorbance at 595 nm) of tubes #16 and #17 obtained by the examiner and shown below, calculate protein concentration of fractions in the examiner's tubes #7 and #8 . Write the protein concentrations of tubes #7 and #8(rounded to two decimal places ) **on the answer sheet (5 POINTS)**.

使用標準曲線和由監考者提供之管＃16和＃17在595 nm的吸光度，

如下所示，計算管＃7和＃8中的蛋白質濃度(記錄至小數點後二位)。 在答案紙上寫下＃7和＃8管的蛋白質濃度（**5**分） 。

| | | |
|---|---|---|
| Tube 16: | Well B9: 0.32 | Well D9: 0.34 |
| Tube 17: | Well B11: 0.41 | Well D11: 0.43 |

## C. Gel retardation assay 凝膠電泳阻滯分析

This assay will be performed with contents of **your** tube #8 (= tube containing drops you collected in the third tube after addition of buffer B).
此分析會使用你的管＃8的內含物來進行（也就是加入buffer B之後收集的第三管）。

1. Dilute contents of tube #8 as follows:
- Mix 10 µl of contents of tube #8 and 40 µl of PBS in tube #18. .
稀釋管＃8的內容如下：
- 取管＃8 的內含物10 µl 和 40 µl PBS 混合置於管＃18中。

2. Tubes #19-25 should be prepared as described below in Table 3. Add components in said order (from top to bottom):
管＃19-25應按下表3所述製備。按所述順序添加組件（從上到下）：

***Table 3***

| **Tube #** | **19** | **20** | **21** | **22** | **23** | **24** | **25** |
|---|---|---|---|---|---|---|---|
| Plasmid DNA | 2 µl | 2 µl | 2 µl | 2 µl | 2 µl | 2 µl | 2 µl |
| Pep, tube #18 | 0 | 3 µl | 7 µl | 0 | 0 | 0 | 0 |
| Pep, tube #8 | 0 | 0 | 0 | 4 µl | 7 µl | 0 | 7 µl |
| PBS | 13 µl | 10 µl | 6 µl | 9 µl | 6 µl | 9 µl | 2 µl |
| DNase buffer | 0 | 0 | 0 | 0 | 0 | 2 µl | 2 µl |
| DNase (0.015 U/ µl) | 0 | 0 | 0 | 0 | 0 | 2 µl | 2 µl |

3. Three minutes after addition of DNase to tubes 24 and 25, add 3 µl gel loading dye to each of tubes #19-25 and mix.
將DNase加入管#24和#25，3分鐘後，再於管＃19-25中加入3 µl 凝膠上樣染料並加以混合。

4. The gel electrophoresis apparatus will be off at this time. Be careful not to press the power button or any other button on the apparatus. Load 15 µl of each of tubes #19-25 consecutively into 7 adjacent wells (from left to right when positive pole is closer to you) of the agarose gel in the electrophoresis apparatus. In the eighth well add 15 µl of DNA size marker which already contains gel loading dye. NOTE: The gel is covered with electrophoresis buffer, therefore add the 15 µl aliquots very gently to the bottom of each well to prevent spill over while loading.
此時，凝膠電泳儀器將被關閉。小心不要按設備上的電源按鈕或任何其他按鈕。從管＃19-25各取15 µl，連續加入瓊脂凝膠的相鄰7個well 中(由左至右，電泳裝置的正極靠近你)。

在第8 well中，加入15 µl 已含有凝膠上樣染料的DNA大小標記物。注意：凝膠用電泳緩衝液覆蓋，將15 µl的樣品非常溫和地加到每個well的底部，以防止溢出。

NOTE: One well of the gel has been left empty.
注意：凝膠中有一個 well 留空。

5. After completion of loading, insert the orange colored photo hood onto the apparatus. Press the power button on the lower right surface of the apparatus to start the electrophoresis. Record time of start of electrophoresis. The two buttons on the upper right are for illumination with high level or low level blue lighting. Press the button for high level lighting. This will enable you to visualize migration of the DNA in the gel real time during electrophoresis because the DNA binding stain is in the gel. Migration should be visualized through the hole on top of the photo hood. The photo hood should not be removed.
樣品裝載於電泳膠完成後，將橙色照相遮罩插入設備。按下右下方的電源按鈕開始電泳，並記錄電泳開始的時間。

右上方的兩個按鈕用於照射高量或低量的藍光。按下按鈕進行高量的照光。此有助於你在電泳過程中即時看到凝膠中DNA的遷移，因為凝膠中已有會與DNA結合的染劑。

應該可從照相遮罩上方的孔看到DNA的遷移。不應移除此照相遮罩。

6. Disconnect the electricity 15 minutes after start of electrophoresis by pressing the power button. Place your green flag in your flag stand to attract attention of lab assistant. He/she will take photo of the gel through the hole of the photo hood (35 POINTS). You may proceed to the theory questions below during the 15 minute interval.
開始電泳15分鐘後，按電源按鈕以切斷電源。

將你的綠旗放在旗架上，以吸引實驗室助理的注意力。他/她將透過照相遮罩的孔拍攝凝膠照片（35分）。

您可以在這15分鐘內繼續回答下面問題。

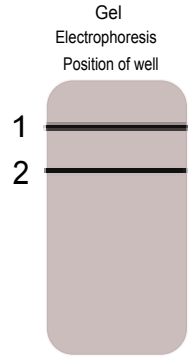
**Questions (15 POINTS in total):**（總共**15**分）：
Indicate if each of the following statements is true or false with “X” in the answer sheet.
在答案紙中用“X” 指出下列敘述是對還是錯。

1. The larger the linearity range of a protein assay, the less one needs to be concerned about the concentration of the sample of interest to be used in the assay (1 **POINT)**.
蛋白質測定的線性範圍越大，越不須要注意欲測定樣品的濃度（1 分）。

2.The effect of Pep on DNase activity on the plasmid DNA is likely to depend on the sequence of the DNA (1 **POINT)**.
Pep對質體DNA上DNase活性的影響可能取決於DNA序列（1 分）。

3. Observation of stained DNA in gel regions above the wells would reflect results of interaction of DNA with high concentrations of Pep **(1 POINT)**.
在wells上方的凝膠區域中，觀察染色的DNA位置將反映出DNA與高濃度Pep 間的相互作用 **(1** 分**)**。

4. Assume the schematic figure shown below represents the electrophoresis pattern of contents of tube 19. The upper band may be circular plasmid DNA in which one phosphodiester bond in one strand has been broken (1 **POINT)**.
假設下圖所示代表管**#19**內含物的電泳圖。上方的條帶可能是環狀質體DNA，其中一條鏈中的一個磷酸二酯鍵已被破壞**(1** 分**)**。



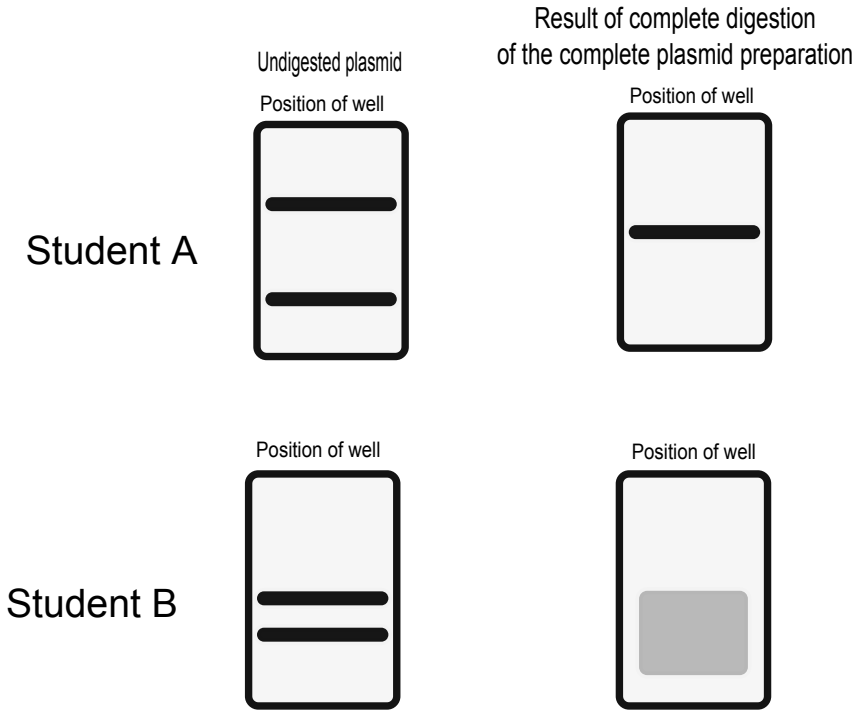

5. Student A attempted to purify a plasmid, and student B attempted to purify another plasmid. Neither plasmid had repetitive sequences. After electrophoresis of the plasmids, both students observed two bands as shown below. Based on known size of the plasmids, the upper band was unexpected. Each performed restriction enzyme digestion on the plasmid preparation under conditions of achieving complete digestion, and subsequently electrophoresed the digestion product. Results are shown below. The map of the plasmids showed that each contained only one recognition site for the enzyme used.

學生A試圖純化質體，學生B試圖純化另一質體，此兩質體沒有重複序列。

在質體電泳後，兩個學生觀察到如下圖所示的兩個條帶。

依據已知的質體大小，上方的條帶是出乎意料的。

每個質體製備後以核酸限制酶進行完全切割後，隨後對切割後的產物進行電泳。結果如下所示。質體圖譜顯示每個質體僅含有一個所用限制酶的識別切割位點。



Indicate if each of the following statements is true or false **in the answer sheet (4 POINTS)**.
在答案紙中指出以下每個陳述是對還是錯（**4** 分）。

a) The unexpected band of student A may be linearized plasmid DNA.
學生A的非預期之電泳條帶可能是直線型的質體DNA。

b) The unexpected band of student A may be circular dimer plasmid DNA.
學生A的非預期之電泳條帶可能是環狀二聚體質體DNA。

c) Electrophoresis pattern of partial digestion product of the plasmid preparation of student A with the same restriction enzyme is expected to produce at most three bands.
在相同限制酶處理下，學生A的質體製備物的部分切割產物的電泳模式，預期至多可產生三個條帶。

d) Electrophoresis pattern of partial digestion product of the plasmid preparation of student B with the same restriction enzyme is expected to produce three bands.
在相同限制酶處理下，學生B的質體製備物的部分切割產物的電泳模式，預期至多可產生三個條帶。

6. To determine the restriction enzyme map of two rare restriction enzymes on a linear DNA molecule of interest, RE1 and RE2, the following experiment was performed. First, samples of the DNA molecule were labeled at their 5' ends and cut separately with RE1 and RE2. The lengths of the fragment products with label are shown in the figure below with **Stars.** Other samples of the DNA in unlabeled form were also cut separately with RE1 and RE2. RE1 digestion products were loaded in a very wide well of a gel and electrophoresed. After electrophoresis, DNA bands on the gel were transferred to a nitrocellulose filter paper by Southern blotting. RE2 digestion products were also loaded in a very wide well of another gel and also electrophoresed. Then DNA bands on this gel were transferred by Southern blotting to the same nitrocellulose filter paper that contained the digestion products of RE1, but transfer was oriented perpendicular relative to the transfer of RE1 restriction fragments. **Circles** show positions of hybridization between RE1 digestion fragments and RE2 digestion fragments.

6. 為了確定兩種稀有限制酶RE1和RE2對目標線型DNA分子的限制酶圖譜，進行以下實驗。

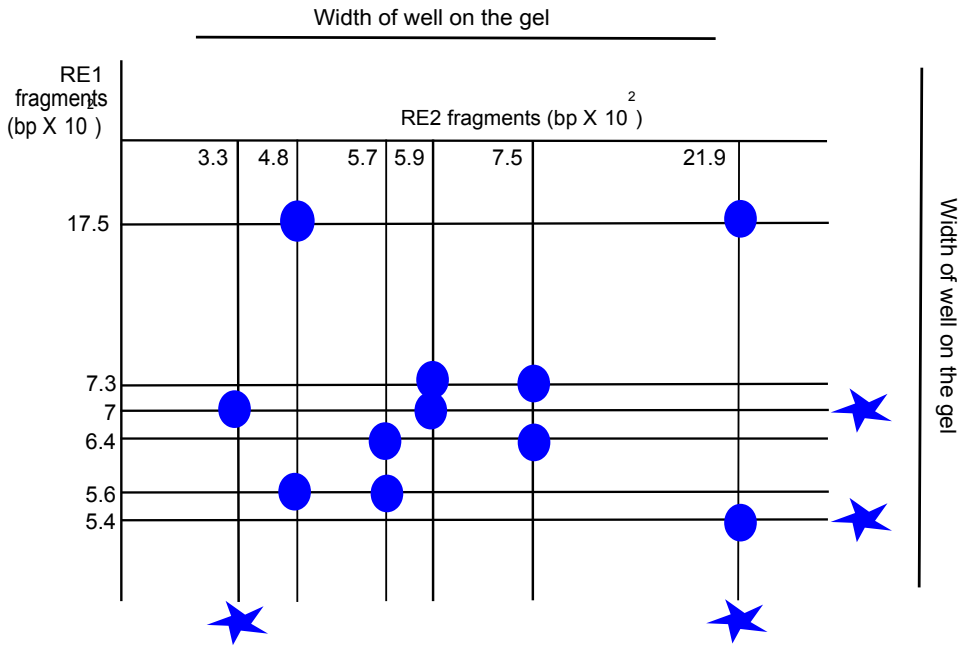
首先，DNA分子的樣品在其5'端被標記，並分別用RE1和RE2切割。

帶有星號標記的片段產物的長度如下圖所示。其他未標記者的DNA樣品也分別用RE1和RE2切割。

將RE1切割產物載入非常寬的凝膠孔中並電泳。電泳後，通過Southern轉漬印跡將凝膠上的DNA條帶轉移到硝酸纖維素濾紙上。

RE2切割產物也加載到另一個凝膠的非常寬的孔中並進行電泳。然後通過Southern轉漬印跡將該凝膠上的DNA條帶轉移到含有RE1切割產物的相同硝酸纖維素濾紙上，但轉漬時，以垂直於RE1的角度來轉移。

圓圈顯示RE1切割片段和RE2切割片段之間雜合後的位置。




Indicate if each statement below is true or false (7 **POINTS)**.
指出下列敘述的對錯（7 分）。

a. Complete digestion of the DNA molecule with RE1 and RE2 would produce 11 fragments.
用RE1和RE2完全切割DNA分子將產生11個片段。

b. The 6.4 X$10^2$ bp and 7.3 X$10^2$ bp DNA fragments produced by digestion with RE1 are adjacent in the undigested DNA molecule.
用RE1切割產生的6.4 X$10^2$ bp和 7.3 X$10^2$bp DNA片段在未切割的DNA分子中彼此相鄰。

c. The 5.6 X$10^2$ bp and 4.8 X$10^2$ bp fragments produced, respectively, by digestion with RE1 and RE2 are overlapping in the undigested DNA molecule.
分別用RE1和RE2切割產生的5.6 X$10^2$ bp和4.8 X$10^2$ bp片段，會在未切割的DNA分子中重疊。

d. The 17.5 X$10^2$ bp and 21.9 X$10^2$ bp fragments produced, respectively, by digestion with RE1 and RE2 overlap in the undigested DNA molecule.
分別用RE1和RE2切割產生的17.5 X$10^2$ bp和21.9 X$10^2$ bp片段，會在未切割的DNA分子中重疊。

e. No single digestion product of one of the restriction enzymes can overlap with three of the digestion products of the other enzyme.
以一種限制酶切割的單一切割產物不會與另一限制酶切割的三種切割產物發生重疊。

**Student Code:**

# ANSWER SHEET - BIOCHEMISTRY & MOLECULAR BIOLOGY

A. Answer to question in Part 2 of Bradford assay:
回答第2部分Bradford assay的問題：

| Protein concentration of Tube #7:<br>管＃7的蛋白質濃度： |
|---|

| Protein concentration of Tube #8:<br>管＃8的蛋白質濃度： |
|---|

B.Answers to questions 1-6.
問題1-6的答案。

| 說明 | 對 | 錯 |
|---|---|---|
| **1** | | |
| **2** | | |
| **3** | | |
| **4** | | |
| **5A** | | |
| **5B** | | |
| **5C** | | |
| **5D** | | |

| | | |
|---|---|---|
| **6A** | | |
| **6B** | | |
| **6C** | | |
| **6D** | | |
| **6E** | | |