27th International Biology Olympiad

July 17-23, 2016
Hanoi, Vietnam

Practical Exam 3 實作實驗三

BIOCHEMISTRY and 生化與 MICROBIOLOGY 微生物學

Total points: 100 總分: 100分
Duration: 90 minutes 時間: 90分鐘

DEAR PARTICIPANTS,參賽者

This exam consists of three experiments:
這部分考試包括三個實驗:

- Experiment 1: Expression, purification and characterization of proteins (40 points)
  實驗一:蛋白質的表現.純化和定性(40 分)
- Experiment 2: Antioxidant activity of coffee extract (30 points)
  實驗二.咖啡萃取物的抗氧化活性(30 分)
- Experiment 3: Lactic acid fermentation (30 points)
  實驗三:乳酸發酵(30分)

Please note the following:注意事項

- Please remember to write your Country and Student code in the given box
  請記得在空格裡寫下你的國家和選手編號..
- Write your answers in the separate Answer Sheet. Only the answers given in the Answer Sheet will be evaluated.
  寫下你的答案在不同的答案卷中,
  只有寫在答案卷中的答案才給分.
- Make sure that you have received all the materials and equipment listed. If any of these items are missing, please raise the Red card immediately.
  確認表列中的材料和器材是否齊全,如果缺少任何東西,請立刻舉起紅色卡片.
- During experiments, ensure to handle equipment properly. Any spilled solutions or broken equipment will not be replenished.
  實驗進行中,請妥善使用器材,任何傾漏的溶液和摔破的器材將不再補充.
- Gel electrophoresis in Experiment 1 must not be performed in the last 30 minutes. You are recommended to do Experiment 1 first.
  不要在最後30分鐘才操作實驗一電泳實驗,建議你先從實驗一開始操作.
- Ensure to obtain spectrophotometer readings to answer the questions in Experiment 2.
  確定拿到光電比色計的讀值,以方便回答實驗二的問題.
- Stop answering and put down your pen immediately when the bell rings at the end of the exam. Enclose the Question Paper, Answer Sheet, and Data printout in the provided envelope.
  當鈴聲響考試結束後,請立刻停止作答,將題目卷.答案卷和列印出的實驗數據放入信封中
- No paper, materials or equipment should be taken out of the laboratory.
  請勿將任何的紙張.材料或器材帶走.

Materials & Equipment

Equipment and Materials for 3 experiments
三個實驗的器材和材料

Name 物品	Quantity 數量
Micropipette P1000 (100-1000 µl) 微量吸管 (P1000)	1 piece 一支
Micropipette P200 (20 – 200 µl) 微量吸管(P200)	1 piece 一支
Micropipette P20 (2 – 20 µl) 微量吸管(P20)	1 piece 一支
Pipette tips for micropipette P1000 吸管尖(P1000)	1 box 一盒
Pipette tips for micropipette P20 and P200 吸管尖(P20和P200)	1 box 一盒
Deionized water (dH_2O) 去離子水	1 bottle 一瓶
Microcentrifuge rack 微量離心機	1 piece 一台
Round plastic container for liquid waste (Liquid waste) 圓筒型塑膠廢液桶(收集廢液)	1 piece 一個
Square plastic container for solid waste (Solid waste) 方形塑膠固體廢棄桶(收集固體廢棄物)	1 piece 一個
Timer 碼表	1 piece 一個
Gloves 手套	1 pair 一雙
Tissue paper 面紙	1 box 一盒
Glue 膠水	1 tube 一盒
Labels of student code 選手號碼標籤	5 pieces 5張
Red card 紅色卡片	1 piece 一張
Green card 綠色卡片	1 piece 一張
Calculator計算機	1 piece 一台
Marker 蛋白質分子量指標	1 piece 一管
Goggles 護目鏡	1 piece 一個

For Experiment 1
實驗一器材

Name 物品	Quantity 數量
SDS-PAGE electrophoresis gel tank and power supply SDS-PAGE 電泳槽和電源供應器	1 set 一組
Gel comb 電泳齒梳	1 piece 一個
Gel container (with student code) 電泳膠容器(有標示選手編號)	1 piece 一個
Microcentrifuge tubes 1.5mL 微量離心管 1.5mL	10 piece 10個
Polyacrylamide gel cassette assembled in the electrophoresis gel tank Polyacrylamide 電泳膠片卡匣裝配於電泳槽內	1 piece 一個
Magenta microcentrifuge tube with 2X SDS-PAGE loading buffer (Buffer) 裝有2X SDS-PAGE loading buffer(Buffer) 的紫紅色離心管	1 piece 一管
Yellow microcentrifuge tube with protein marker (M) 裝有 protein marker(M)的黃色離心管	1 piece 一管
Microcentrifuge tube with BL21 cells without IPTG (NO_IPTG) 離心管內裝不含IPTG的 BL21細胞(NO_IPTG)	1 piece 一管
Microcentrifuge tube with BL21 cells with IPTG (IPTG) 離心管內裝含有IPTG的BL21細胞	1 piece 一管
Microcentrifuge tube with pellet of cell extract obtained from centrifugation of homogenized BL21 cells with IPTG (Pellet) 離心管內裝有IPTG處理過的BL21細胞經均質化後再經離心的細胞萃取沉澱物(Pellet)	1 piece 一管
Microcentrifuge tube with supernatant of cell extract obtained from centrifugation of homogenized BL21 cell with IPTG (Super) 離心管內裝有IPTG處理過的BL21細胞經均質化後再經離心的細胞萃取上清液(Super)	1 piece 一管
Microcentrifuge tube with purified proteins (Puri-P) 離心管內裝有純化的蛋白質(Puri-P)	1 piece 一管
Falcon tube (green cap) with 30 mL of SDS-PAGE staining solution (STAIN) 綠色蓋子離心管裝有30 mL 的 SDS-PAGE 染液	1 piece 一管

For Experiment 2 實驗 2

Name 名稱	Quantity 數量
96-well microplate with student code (do not touch the bottom of the plate) 96孔微量盤，標示學生代碼(勿觸摸盤底正下方)	1 piece 1個
Blue microcentrifuge tube with 300 µl of 1 mg/mL ascorbic acid solution (AA) 藍色微量離心管(AA)，裝有 1 mg/mL 抗壞血酸 300 µl	1 piece 1管
Blue microcentrifuge tube with 300 µl of 5 mg/mL coffee extract (CC) 藍色微量離心管(CC)，裝有 5 mg/mL 咖啡萃取液 300 µl	1 piece 1管
Brown bottle with 15 mL of 0.2 mM DPPH solution (DPPH) 棕色瓶(DPPH)，裝有 0.2 mM DPPH 溶液 15 mL	1 piece 1瓶

For Experiment 3 實驗 3

Name 名稱	Quantity 數量
Burette containing 25 mL of 0.1M NaOH solution with a stand and a clamp 滴定管附支架和夾子，裝有 0.1M NaOH溶液 25 mL	1 set 1組
Magnetic stirrer 電磁攪拌器	1 piece 1個
Stirring bar 攪拌棒	1 piece 1個
Forceps 鑷子	1 piece 1個
Measuring cylinder (10 mL) 10 mL 量筒	1 piece 1個
Measuring cylinder (25 mL) 25 mL 量筒	1 piece 1個
Beakers (100 mL) 100 mL 燒杯	8 piece 8 個
Hanna portable pH meter and a screw driver 攜帶式 pH儀，和一個螺絲起子	1 piece 一個
Rinsing water bottle (H_2O) 水洗瓶 (H2O)	1 piece 一個
Bottle with pH 4.01 buffer (pH4.01) pH 4.01 緩衝液 (pH4.01)	1 piece 1瓶
Bottle with pH 7.01 buffer (pH 7.01) pH 7.01 緩衝液 (pH7.01)	1 piece 1瓶
Falcon tubes with 15-30 mL supernatant of culture broth (samples A0, A2, A3 and A5) 裝在離心管中15-30 mL的上清液樣本 (A0、A2、A3、A5)，	4 piece 4管

EXPERIMENT 1. EXPRESSION, PURIFICATION AND CHARACTERIZATION OF PROTEINS (40 POINTS).
實驗 1. 蛋白質的表現、純化和分析

Introduction 介紹

H and B proteins are two important proteins of Aeromonas hydrophilas. To study them, a scientist wanted to co-express them in E. coli. For this, gene b was cloned into multiple cloning site 1 (MCS-1) and gene h in MCS-2 of expression vector p1 (Fig.1.1). The obtained p1-b-h vector was transformed into E. coli and protein expression was induced by IPTG (isopropyl β-D-1-thiogalactopyranoside). The proteins were then purified by affinity chromatography, by which a protein containing 6xHis-tag binds to the nickel column. Expression and purification were finally evaluated by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis), a method for separation of proteins based on their molecular weight. Note that H is smaller than B.
H 和 B 蛋白是 Aeromonas hydrophilas 細胞內兩種重要的蛋白質,為了研究目的,一位學者想要在 E. coli. 細胞內同時表現這兩種蛋白質,為此,他將b基因選殖進入表現載體p1 的MCS-1選殖位中;h基因則選殖進入p1載體的MCS-2 選殖位中(Fig1.1).再將這個重組過後的表現質體 p1-b-h 轉殖進入E. coli 細胞中,並利用 IPTG (isopropyl β-D-1-thiogalactopyranoside)來誘導蛋白質的表現.接下來利用親和力的層析法純化這些蛋白質,其純化的原理是藉由一個蛋白質含有6個組氨酸標籤(6xHis-tag)可與鎳管柱(nickel column)結合,這些表現和純化的蛋白質最後經SDS-PAGE電泳分析,其原理是利用不同的分子量來將蛋白質分離,但請注意H蛋白的分子量較B蛋白小.

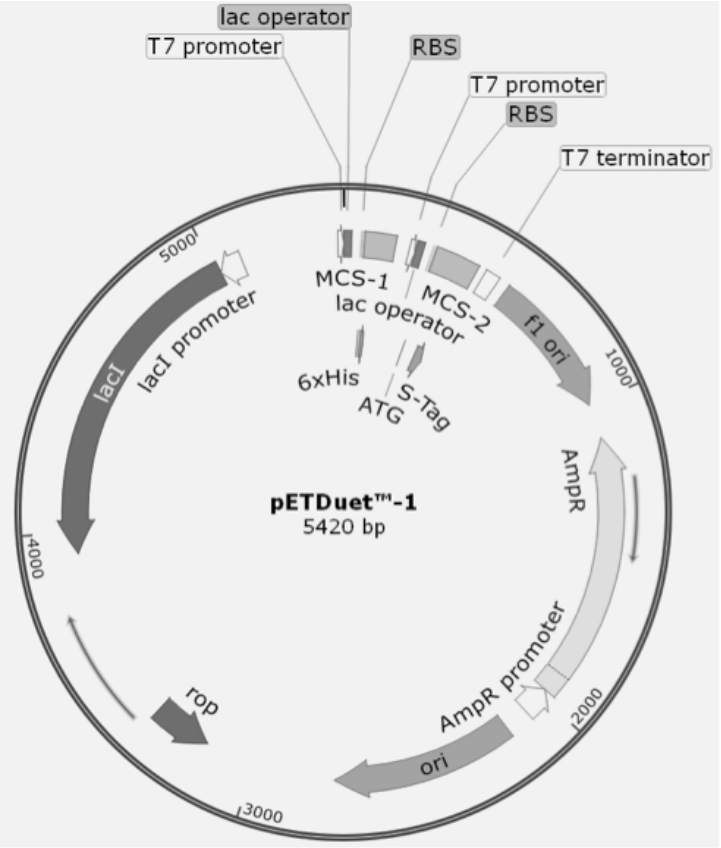


Fig.1.1. Overview map of plasmid p1 expression vector
表現載體p1的基因構造圖
(ori: origin, rop: repressor of primer, AmpR: ampicillin resistant)
(ori: 複製起始點, rop: 引子的抑制蛋白基因, AmpR: 抗抗生素 ampicillin 基因)

A single colony of E. coli containing p1-b-h vector was cultured in 50 mL of LB medium. The culture was grown at 37ºC until the OD600 reached 0.6. In order to analyze the expression and purification of the recombinant proteins, the scientist has collected the following cell and protein samples:
一個含有重組過後的表現載體 p1-b-h vector 的單一的E. coli 菌落被挑選出來,並將此菌落中的細菌培養在 50 mL 的 LB 培養基中,在 37ºC 下培養至細菌濃度達到 OD600 吸光值達 0.6,為了方便分析這些重組蛋白質的表現和純化,這位學者收集了以下的細胞和蛋白質樣本.

- NO_IPTG. One mililiter of the culture was transferred to a – tube, grown at 20ºC for 16 hours (OD_{600} = 2.4) and then centrifuged. The supernatant was discarded and the cell pellet was resuspended in 50 µl H_2O and then mixed with 50 µl 2X SDS-PAGE loading buffer to yield 100 µl sample.
  NO_IPTG. 將 1 mL 的細菌培養液倒入標示 a的 離心管(a-tube)中,在 20ºC 下培養 16 小時濃度達到 OD_{600} = 2.4,將菌液離心,倒掉上清液後,將細胞沉澱物回溶於 50 µl H_2O 中,再與 50 µl 的 2X SDS-PAGE loading buffer 混合後,以獲得總體積為 100 µl 的樣本

In the remaining 49 mL culture, protein expression was induced by adding IPTG. The culture was further grown at 20ºC for 16 hours.
剩下的49 mL 的細菌培養液,用以進行IPTG 誘導實驗，在細菌內表現蛋白質,隨後讓細菌繼續在 20ºC 下生長 16 小時.

- IPTG: One milliliter of the culture with IPTG (OD_{600} = 1.4) was centrifuged. The supernatant was discarded and the cell pellet was resuspended in 50 µl H_2O and then mixed with 50 µl of 2X SDS-PAGE loading buffer to yield 100 µl sample.
  IPTG: 將 1 mL 經 IPTG 處理過的細菌培養液(OD_{600} = 1.4)離心,倒掉上清液後,將細胞沉澱物回溶於 50 µl H_2O 中,再與 50 µl 的 2X SDS-PAGE loading buffer 混合後,以獲得總體積為 100 µl 的樣本.

The remaining 48 ml culture was centrifuged, the supernatant was discarded and the cell pellet resuspended into 2 mL of nickel binding buffer. The cell suspension was lysed and subsequently centrifuged. Both pellet and supernatant were collected.
將剩下的 48 mL 的細菌培養液離心,倒掉上清液後,將細胞沉澱物回溶於 2 mL 的 nickel binding buffer 中,將此細胞懸浮液打碎裂解,隨後加以離心.請注意離心後的沉澱物和上清液兩者都要收集
.

- Pellet: The pellet obtained from the IPTG-cell lysate was resuspended into 2 mL buffer and then mixed with 2 mL of 2X SDS-PAGE loading buffer (pellet stock solution).
  Pellet:將 IPTG 處理過的細胞裂解液經離心後所獲得的細胞沉澱物,回溶於 2 mL buffer 中,再與 2 mL 的 2X SDS-PAGE loading buffer 混合後,以獲得 pellet stock solution.
- Super: 10 µl of supernatant obtained from IPTG-cell
  lysate was mixed with 10 µl of 2X SDS-PAGE loading buffer
  Super:取 IPTG 處理過的細胞裂解液經離心後所獲得的上清液 10 µl ,與 10 µl 的 2X SDS-PAGE loading buffer 混合.
- Puri-P: The rest of the supernatant was loaded onto the nickel column for protein purification. The purified proteins were eluted from the column using 2 mL elution buffer.10 µl of purified protein was mixed with 10 µl of 2X SDS-PAGE loading buffer.
  Puri-P: 將剩下的上清液注入鎳管柱(nickel column)中進行蛋白質純化,利用 2 mL 的 elution buffer 將純化的蛋白質洗出,再將 10 µl 的純化蛋白質與10 µl 的 2X SDS-PAGE loading buffer 混合.

All samples for SDS-PAGE analysis were boiled at100ºC for 5 min.
將所有要進行 SDS-PAGE 分析的樣本於 100ºC 中加熱 5 min.

Design your SDS-PAGE experiment to analyze protein expression.
設計你的SDS-PAGE實驗以分析蛋白表現

The standard final concentration of total protein for SDS-PAGE analysis must be equal to $5x10^6$ cells/µl. At first, calculate the concentration of cells in each sample, knowing that OD_{600} value of 1 corresponds to $8x10^8$ cells/mL and take into consideration the dilution of each sample during the procedure.
將進行 SDS-PAGE 分析的全部蛋白質的最終標準濃度必須相當於 $5x10^6$ 細菌細胞/µl,首先,計算每個樣本中的細胞數目,吸光值 OD_{600}=1 相當於 $8x10^8$ cells/mL,在實驗過程中請特別注意每種不同樣品的稀釋倍率.

Q.1.1 (6 POINTS)
問題.1.1(6分)

Calculate and fill the volume (µl) of samples in the table in the Answer Sheet. Use one decimal place.
計算並填滿答案卷中本表的樣本體積 (µl) ,數字呈現到小數點後一位.

Procedures
實驗步驟

1. ased on the table above, prepare all SDS-PAGE samples in empty microcentrifuge tubes provided. Mix each sample by pipetting up and down 4-5 times.
根據上表,將所有將進行 SDS-PAGE 分析的樣本混合在空的離心管中,並吸排混合4-5次.

   After completing this step please raise the Green card. An assistant will guide you to the loading area and help to stick your student code to the gel tank..
當你完成這個步驟時,請舉起綠色卡片,有一位助理將引導你至loading area,並將選手編號黏貼於電泳槽上.

2. Load 20 µl of each sample on SDS-PAGE gel. Samples must be loaded in order from tube 1 to 6. To load a sample, use the P20 micropipette with tip to withdraw 20 µl of a sample, and carefully place the tip on the top of the well (Fig.1.2).
每個樣本取 20 µl 注入SDS-PAGE 電泳膠片中,記得必須依照離心管 1 到 6 的順序注入樣本,請使用 P20 微量吸管吸取樣本 20 µl,再小心地將吸管尖置入每個 well 的頂端.(圖.1.2).

Fig.1.2. Sample loading on SDS gel

3. The assistant will run the SDS-PAGE for 20 min and tell you to set your timer for 20 min.
助理將進行 SDS-PAGE 電泳 20 min,且告訴你將碼表設定在 20 min後.

   You can do another experiment while running SDS-PAGE. After 20 min, please raise the Green card to inform an assistant to return the SDS-PAGE gel to you.
當 SDS-PAGE 電泳實驗進行時.,你可以操作其他實驗,二十分鐘後,請舉起綠色卡片告知助理將你的 SDS-PAGE 膠片取回.

4. Remove the SDS-PAGE gel from the plastic cassette using the gel comb as in the diagram below (Fig.1.3.) and put the gel into the gel container.
使用如下圖所示的齒梳,從塑膠卡匣中取下你的 SDS-PAGE 膠片.將它放置於膠片容器(gel container)中.

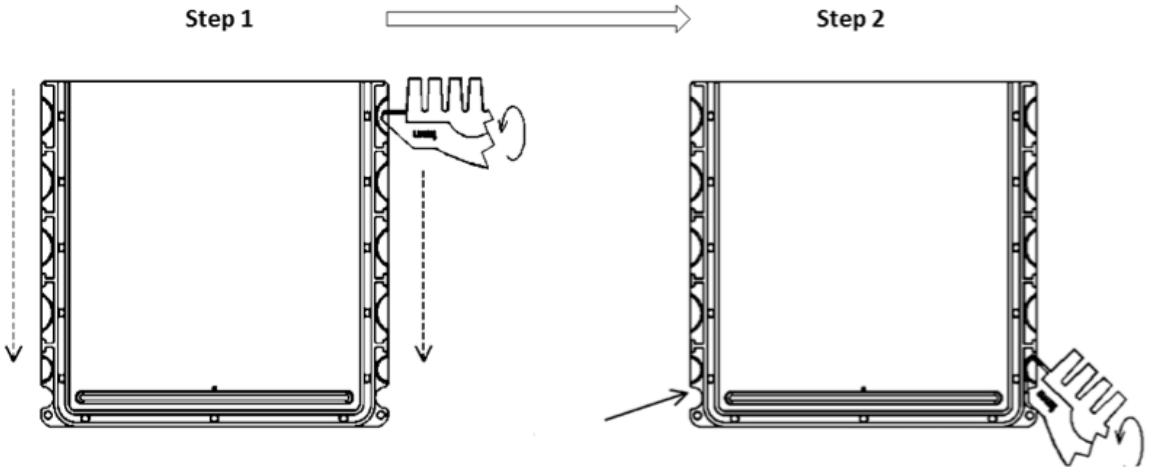


Fig.1.3. Remove the SDS-PAGE gel from the plastic cassette
圖.1.3.從塑膠卡匣中取下 SDS-PAGE 膠片

Step 1. Crack open cassette sides by inserting the comb's slanted edge into each of the notches around the cassette and twisting firmly. Starting with the notches at the top, move down each side of the cassette.
步驟一.將齒梳斜面的尖端部位插入塑膠卡匣四周鋸齒狀的凹洞內,並穩固地旋轉,敲開兩側的塑膠卡匣,順序是從兩側上方的鋸齒狀凹洞開始,逐一往下操作(如圖中 step 1).

Step 2. After the sides are open, place the comb's slanted edge at a 45-degree angle between the plates at each bottom corner and twist firmly.
步驟二.當卡匣兩側已經敲開後,將齒梳斜面的尖端部位以45度角插入兩側底部角落的凹洞,並且穩固地旋轉.(如圖中 step 2)

Step 3. Gently separate the two pieces of the cassettes.
步驟三.緩慢地分開兩塊塑膠卡匣.

5. Add 40 mL of staining solution (STAIN) into the gel container and rock on the rocker for 10 min.
   將 40 mL 染劑(STAIN)加入膠片容器中,再置於搖動器上搖動 10 min.
6. Discard the staining solution from the gel container and rinse the gel 3 times using deionized water.
   倒掉膠片容器中的染劑,利用去離子水潤洗膠片三次.

When finished, raise the Green card to ask an assistant to take photo of the gel.
完成後,舉起綠色卡片請助理幫忙將膠片照相.

Q.1.2. SDS-PAGE RESULT (10 POINTS)
問題.1.2.SDS-PAGE 結果 (10 分)

After obtaining the photo of SDS-PAGE gel, stick it to the place given in the Answer Sheet.
拿到照片後,將照片黏貼於答案卷的框框中.

Q.1.3 (4 POINTS)
問題.1.3(4 分)

Based on the information provided in Fig.1.4A below, plot the molecular weight of at least five marker proteins versus their relative migration-Rf values on the graph paper provided in the Answer Sheet. (Rf: distance migrated by protein/distance migrated by dye front)
根據下圖.1.4A所提供的訊息,在答案卷的方格圖紙中畫出至少五種已知蛋白質分子量指標(marker proteins)的分子量與它們移動-Rf值的關係圖.(Rf: 蛋白質移動的距離/前方loading dye移動的距離)

Q.1.4. (4 POINTS)
問題.1.4.(4 分)

Use the graph from Q.1.3 and the SDS-PAGE gel to estimate the molecular mass of H and B proteins.
利用問題.1.3 的圖形和 SDS-PAGE 膠片結果,估算出 H 和 B 蛋白的分子量.

任務

Q.1.5 (4 POINTS)
問題.1.5(4 分)

Based on the SDS-PAGE result, indicate if each of the following statements is true or false.
Mark "√" for True or False statements in the Answer Sheet.
根據 SDS-PAGE 的結果,判斷下列敘述何者正確或錯誤,並在答案卷上打 "√" .

A H protein is over expressed in LB media with IPTG.
在加入 IPTG 處理後的 LB培養基中,B 蛋白有過量表現的現象.

B B protein is completely soluble in the nickel binding buffer.
B 蛋白可完全溶解在 nickel binding buffer中.

C H and B protein interact with each other.
H 蛋白和 B 蛋白彼此之間有交互作用.

D Majority of recombinant proteins was bound to the nickel column.
大多數的重組蛋白質可與鎳管柱(nickel column)結合.

Q.1.6. (4 POINTS)
問題1.6.(4 分)

Based on detailed restriction map of the p1 expression vector (Fig.1.5), indicate if each of the following statements is True or False. Mark "√" for True or False statements in the Answer Sheet.
根據 p1 表現載體的詳細限制酶切點的圖譜(圖.1.5.),判斷下列敘述何者正確或錯誤,並在答案卷上打 "√" .

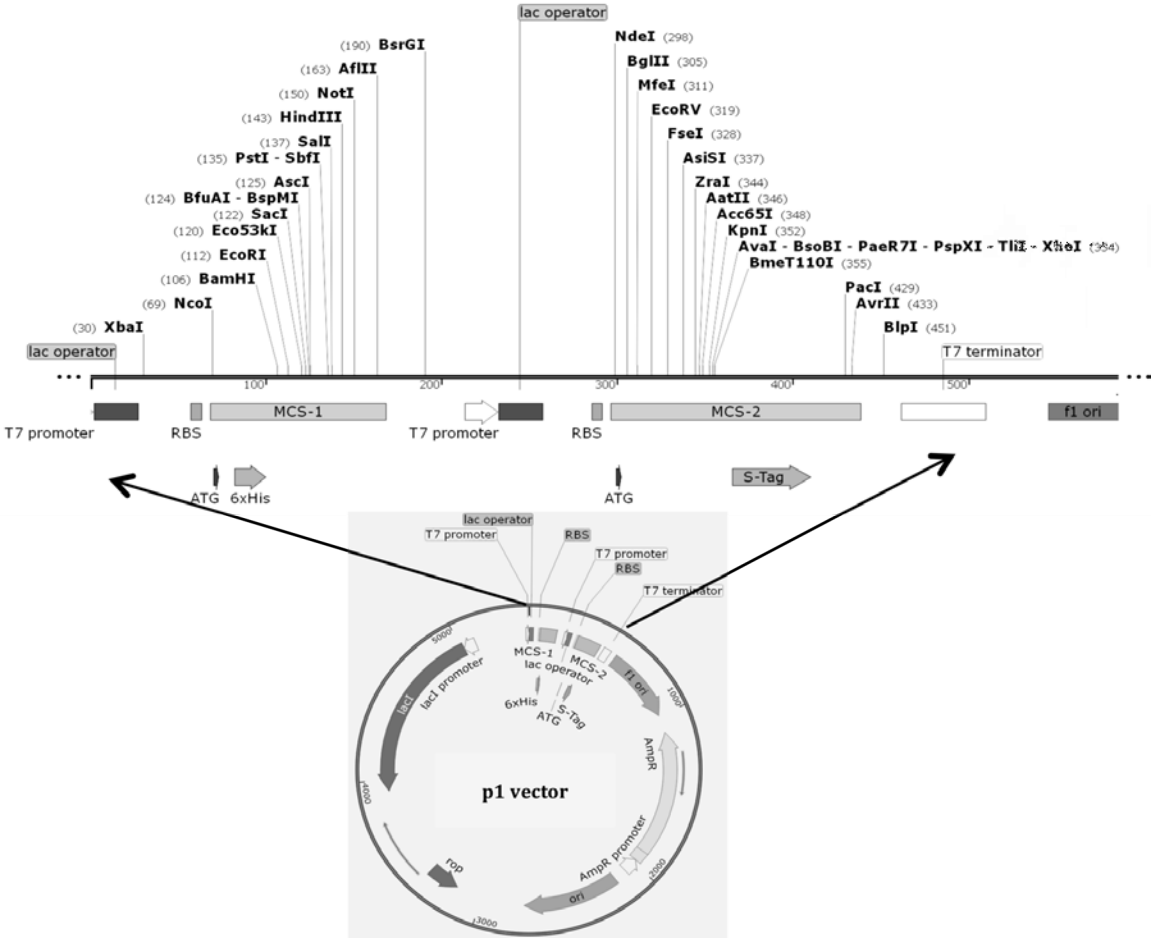


Fig.1.5. Detailed restriction map of p1 expression vector (RBS: ribosome binding site)
圖.1.5. p1 表現載體的詳細限制酶切點的圖譜(RBS: 核糖體結合位置)

任務

A SalI and BamHI can be used to insert b gene into MCS-1.
限制酵素SalI 和 BamHI 可用來將 b 基因選殖進入 MCS-1中.

B Gene h and b should be cloned in the same orientation to be expressed simultaneously.
h 基因與 b 基因應該是以相同的方向被選殖進入載體中,才能同時表現出蛋白質

C Gene h and b should be in the same reading frame to be expressed simultaneously.
h 基因與 b 基因應該是使用相同的讀序框,才能同時表現出蛋白質

D To maintain the plasmid, ampicillin should be added to the culture medium.
為了保留細菌中選殖後的質體,抗生素 ampicillin 必須被加入培養基中

In order to characterize the oligomeric states of H and B proteins, 3 protein samples were prepared: (1) H protein; (2) H and B proteins obtained from the above experiment; (3) B protein. Samples 1 and 2 were transparent but in sample 3 most of the protein was precipitated. Samples 1 and 2 were then loaded on a gel-filtration column. The obtained profiles are shown in Fig.1.6A. The sizes of reference molecule on a gel-filtration column are shown in Fig.1.6B.
為了方便了解 H 和 B 蛋白的寡聚合狀態,以下三種蛋白質的樣本被製備:(1)H 蛋白；(2)上述實驗獲得的 H 和 B 蛋白；(3)B 蛋白。結果顯示樣本 1 和樣本 2 都是澄清透明的,但樣本 3 大部分的蛋白質卻沉澱下來。接下來,將樣本1 和樣本 2 注入膠體-過濾層析管柱中.管柱層析所得到的結果如圖.1.6A 所示, 圖1.6B 中T. F. A. O. CA 和 R 為六種不同分子量的已知參考蛋白質.

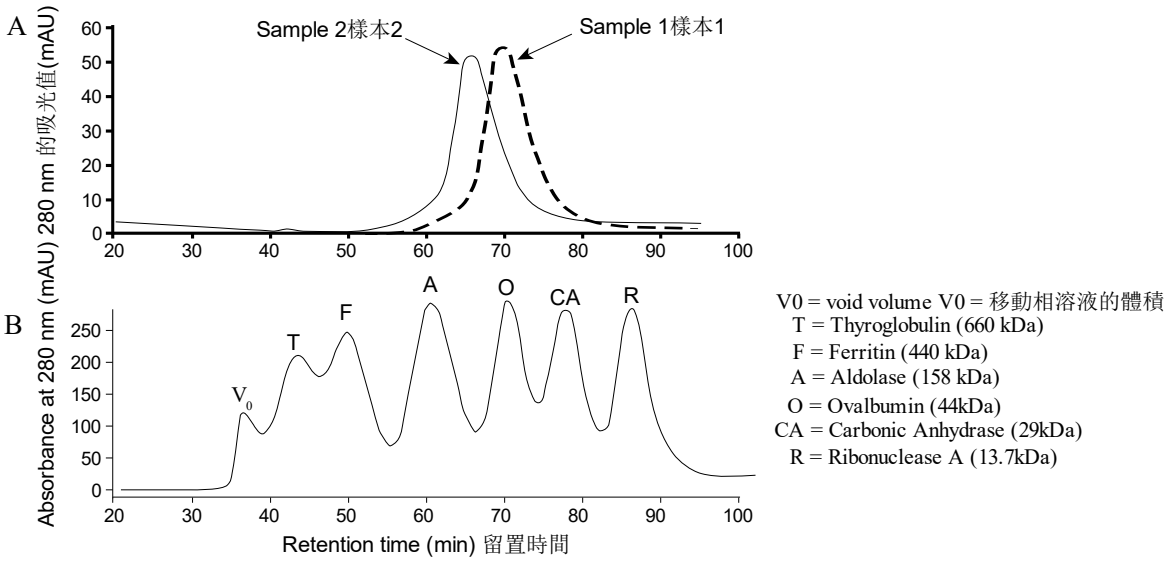


Fig.1.6. Gel filtration analysis of H and B proteins
圖.1.6B, H 和 B 蛋白的膠體-過濾管柱層析的結果
(A) Chromatogram of sample 1 (dashed line) and 2 (solid line)
(A)樣本 1(虛線表示)和樣本 2 (實線表示) 的層析結果'
(B) Chromatogram of reference molecules
(B)層析分析使用已知分子量的參考蛋白質

Q.1.7. (4 POINTS)
問題.1.7.(4 分)

Calculate and report the relative size of the proteins corresponding to gel filtration peaks from sample 1 and 2 on the table in the Answer Sheet.
利用樣本 1 和樣本 2 經膠體-過濾管柱層析結果的波峰位置,估算它們的相對分子量大小,並寫在答案卷的表格中

Q.1.8. (4 POINTS)
問題1.8(4分)

Indicate if each of the following statements is True or False. Mark "√" for True or False statements in the Answer Sheet.
判斷下列敘述何者正確或錯誤,並在答案卷上打 "√" .

A H protein exists as monomer
H蛋白是以單體(monomer)的型式存在

B H and B probably exist as heterodimer
H 和 B 蛋白應該是異型二聚體(heterodimer)的型式存在.

C H protein helps to stabilize B protein.
H 蛋白幫助穩定 B 蛋白的結構.

D In native gel-filtration column analysis, retention time of a protein is proportional to their molecular weight.
在膠體-過濾層析中，蛋白質的留置時間和它們的分子量成比例關係.

EXPERIMENT 2. ANTIOXIDANT ACTIVITY OF COFFEE EXTRACT (30 POINTS)
咖啡萃取物的抗氧化活性（30 分）

Introduction簡介

Biological oxidation produces reactive oxygen radicals that can cause serious damage to cells. Antioxidants are molecules that can scavenge radicals and thus inhibit oxidative reactions. Antioxidants include reducing agents such as thiol compounds, ascorbic acid and phenolics. Coffee, prepared from roasted coffee beans, is a potential source of antioxidants.
生物氧化作用會產生對細胞嚴重傷害的活性氧自由基。抗氧化劑是可以清除自由基而抑制氧化反應的分子。抗氧化劑包括還原劑如硫基化物、抗壞血酸及酚類物質。由烘焙過的咖啡豆所製備的咖啡是一種潛在的抗氧化劑來源。

In this experiment, a 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, in which DPPH is reduced and looses its purple color, is performed. SC_{50} value (scavenging capacity) is commonly used for evaluation of antioxidant activity. This value represents the concentration of sample which scavenge 50% of DPPH radicals. Absorbance of DPPH will be measured at the wavelength of 517 nm. Absorbance of blank is assumed negligible. Absorbance of control (without scavenger, Ac) and sample (As) will be used to calculate scavenging percentage (SC%) for each concentration of samples as:
本實驗使用2,2-二苯基-1-苯肼 (DPPH) 清除測定法，主要測定因DPPH的減少所失去其紫色的程度。常用SC_{50}值（代表清除能力）評估抗氧化的活性，此值表示清除50% DPPH自由基的樣品濃度，在 517 nm測量DPPH自由基的吸光度。假設空白組的吸光度可忽略不計，控制組（無清除劑，Ac）和樣本組（As）的吸光度則用來計算每一樣本濃度的清除百分比（SC %）如下：

$$SC\% = (Ac - As) \times 100/Ac$$

A plot will be created based on the logarithm of concentration series of samples and corresponding scavenging percentage, from which SC_{50} value will be calculated.
根據一系列不同濃度樣品的對數值，和其對應的清除百分比作圖，從而計算 SC_{50} 的數值。

In this experiment, beans of a Vietnamese coffee variety (Coffea canephora) will be investigated for antioxidant activity. Coffee bean powder (1g) was suspended in deionized water at 80°C for 30 min, then filtered and water was added to a final volume of 200 mL of extract solution.
本實驗研究越南品種咖啡豆（Coffea canephora）的抗氧化活性。將咖啡豆粉 (1 g) 浸在80°C的去離子水中，30 分鐘後將懸浮液過濾，再加水成為最終體積為200 毫升的萃取液。

Procedure and questions
步驟與問題

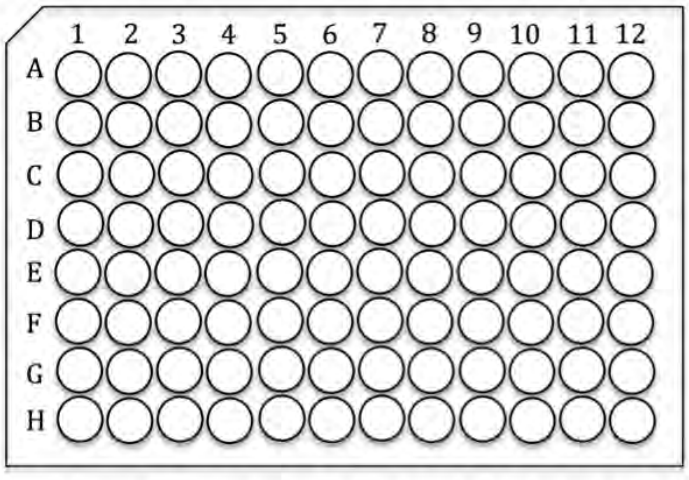


Fig 2.1 96-well microplate
96孔微孔盤

The 96- well microplate above can be used to perform a serial dilution. Positions on this plate are indicated by a letter (A – H) and a number (1 – 12) specifying rows and columns respectively.
上圖的96孔微孔盤可用於進行系列稀釋，盤上的位置是由一個字母(A-H)和一數字(1-12)組成，分別表示在這盤上的特定行和列。

1. Use your micropipette to prepare 4 solutions of ascobic acid (AA1-AA4 in wells A1 – A4 in the 96-well microplate) and 4 solutions of coffee extract (CC1-CC4 in wells A6 – A9 in the 96-well microplate) by serial dilution with a dilution factor of 2, to achieve a lowest concentration of 0.025 mg/mL and 0.625 mg/mL, respectively. The volume of each solution produced should be 200 uL before further dilution is performed. 1.
   用微量吸管以2倍系列稀釋方式，製備4個抗壞血酸溶液（AA1到AA4，分別置於96孔微孔盤的A1-A4位置）及4個咖啡萃取物的溶液（CC1 到 CC4，分別置於96孔微孔盤的A6–A9位置），使抗壞血酸和咖啡萃取物的最低濃度分別為 0.025 mg/mL和 0.625 mg/mL。在每次進行進一步稀釋前，各溶液的體積應為200 µl。
   Note: If an error is made in loading any of these wells use wells H1-H4 for ascobic acid solutions AA1 to AA4 and/or wells H6 – H9 for coffee extract solutions CC1 – CC4.
   注意：若有任何步驟錯誤，利用H1-H4的位置分別放抗壞血酸溶液AA1到AA4，用H6到H9位置分別放咖啡萃取溶液CC1 – CC4。

Q.2.1 (4 POINTS分)

Fill in the table in the Answer Sheet what you calculated for preparing ascorbic acid and coffee extract dilutions.
在答案卷的表中填寫你製備抗壞血酸和咖啡萃取物稀釋液的計算。

2. Pipette 20 uL of ascorbic acid solution and/or coffee extract solution from each well in row A to the corresponding wells in rows B, C and D. If an error is made during this step, the procedure may be repeated using the corresponding wells in rows E, F and G.
   從A列的每個孔中吸取20 uL的抗壞血酸溶液和/或咖啡萃取液，移置到B、C 和D列相對應的孔中。如果此步驟發生錯誤，則使用E、F和G列相對應的孔。

3. Pipette an aliquot of 20 µl H_2O into wells B11, C11 and D11.
   吸取20µl 的H_2O到B11，C11和D11孔中。
4. Pipette 180 µl of DPPH solution into all wells prepared in steps 2 and 3.吸取180µl 的DPPH溶液到所有在步驟2及3所製備的孔中。
5. Cover the plate with the lid and incubate at room temperature for 10 min and set the timer.
   以盤蓋蓋住此盤，設置定時器並在室溫下孵育10分鐘。

After completing this step, raise the Green card for the assistant to help you in measuring absorbance on a microplate reader and return your data printout.完成此步驟時, 舉起綠色卡片，請助理幫你用微孔盤讀標儀測量吸光度，並將列印出的資料交給你。

Q.2.2 (5 POINTS分)

Calculate the logarithm ($\log_{10}$) of ascorbic acid and coffee extract concentration and fill in the table in the Answer Sheet (all numbers are rounded to 2 decimal places). You can use your calculator to calculate common logarithm value following steps below:
計算抗壞血酸和咖啡萃取物濃度的對數值($\log_{10}$)，填寫在答案紙的表中（所有數字都四捨五入至2位小數）。你可以用計算機以下列步驟計算常用對數值：

- Press the ON key to turn on the your calculator
  按ON鍵打開計算機
- Press the four keys SHIFT, CLR, 2, and = to return to calculation mode.
  按SHIFT, CLR, 2, = 等四鍵返回到計算模式
- Press the log key 按log鍵
- Enter the number 輸入數字
- Press the = key 按等號 = 鍵

Calculate the mean absorbance for each dilution, the scavenging percentage for each sample and fill in the table in the Answer Sheet.
計算每個稀釋的平均吸光值，每個樣本的清除百分比，填寫在答案卷上。

Q.2.3. (5 POINTS分)

Use the calculated value to plot a linear curve on scavenging percentage versus logarithm ($\log_{10}$) of ascorbic acid concentration in the grid lines given in the Answer Sheet.
用計算出的數值，在答案卷的格線上做清除百分比與抗壞血酸濃度對數($\log_{10}$)的線性曲線。

Q.2.4. (5 POINTS分)

Calculate SC_{50} value of ascorbic acid and coffee extract and fill in the table in the Answer Sheet (You can plot a linear curve for coffee extract in the grid lines given in Q.2.3 but this curve will not be scored).
計算抗壞血酸及咖啡萃取物的 SC_{50}，填寫在答案卷的表中（你可以在Q.2.3的格線中繪製咖啡萃取物的線性曲線，但這條曲線不會被記分）。

Q.2.5. (3 POINTS分)

Using the same protocol, SC_{50} value of extracts of some coffee varieties were collected as follows:
使用相同的流程，收集一些品種的咖啡萃取物其fu6SC_{50}的數值如下：

Coffee extract咖啡萃取物	SC_{50}
X	3.8 mg/mL
Y	2.6 mg/mL

Compare antioxidant activity of different coffee ben types including the one in this experiment (Z) and arrange them in order from the strongest to the weakest and fill in the given space in the Answer Sheet.
比較不同咖啡豆類型的抗氧化活性，包括此實驗中的(Z)，將其從最強到最弱排序，填寫在答案卷上的指定處。

Q.2.6. (4 POINTS分)

Assume that in your experiment the absorbance of all the mixtures of different diluted coffee extracts and DPPH were similar and negligible. Indicate if each of the following statements is true or false.
假設在你實驗中不同稀釋咖啡萃取物所有混合物的吸光度和 DPPH 相似，均可忽略不計。判斷下列各敘述是對或錯。

Mark “√” for True or False statements in the Answer Sheet.
判斷答案卷上敘述的對錯並標記"√"。

A Antioxidant activities of the diluted coffee extracts are negligible. 稀釋的咖啡萃取物其抗氧化活性可以忽略

B To obtain more accurate determination of antioxidant activities, another experiment with higher concentration samples needs to be carried out.
若要更準確定量抗氧化活性，需要用更高濃度的樣本進行另外一個實驗。

C Activity of antioxidant enzymes in coffee extract resulted in the above result.
咖啡萃取物中抗氧化酵素的活性造成以上結果

D If NADH is added in the wells, there will be no change in the assumed absorbance value.
如果在孔中添加NADH，假定的吸光值不會改變。

Q2.7. (4 POINTS分)

A student measured antioxidant activity of a sample using the same protocol as you have done (Protocol A) and a different protocol (Protocol A*) which was modified from Protocol A. The result is shown in the following table.
一名學生用與你相同的流程 (流程 A)及修改過後的不同流程 (流程A *)，測量一樣本的抗氧化活性，結果如下表。

	Protocol 流程A	Protocol 流程A*
SC_{50} (mg/mL)	1.95	3.9

Which of the following changes could result in a higher value of SC_{50}?
下列何種改變會造成更高的SC_{50}數值？

Indicate if each of the following statements is true or false. Mark “√” for True or False statements in the Answer Sheet.
判斷下列各敘述的對錯，在答案卷上標記"√"。

A The student has used 0.1 mM DPPH for Protocol A*.學生在流程A*中用了0.1 mM DPPH

B The student has loaded 10 µl of sample in each well for Protocol A*.學生在流程A*每個孔中注入10 µl 樣本

C After adding DPPH, the student has incubated the 96-well plate for a shorter time than in Protocol A.
加入DPPH之後，學生將96孔微孔盤孵育比在流程A時間短

D The student has used better solvent for antioxidants.
學生為抗氧化劑用了更好的溶劑

EXPERIMENT 3. LACTIC ACID FERMENTATION (30 POINTS)
實作 3. 乳酸發酵(30分)

Introduction簡介

Recently, a scientist isolated a homolactic acid Lactobacillus strain (Lactobacillus sp. VN156) from traditionally fermented mustard in Vietnam. In this experiment, Lactobacillus sp. VN156 was grown in MRS medium. The initial pH of the culture medium was 5.6. Samples were taken at different times during the cultivation for measuring the optical density (OD) of bacterial cells at 600 nm (Fig.3.1). An OD_{600} value of 1 corresponds to $2x10^8$ cells/mL. The samples A0, A2, A3 and A5 are supernatants of collected samples which will be used for analysis of lactic acid production.
最近，在越南有一位科學家從傳統發酵的芥末中分離出單乳酸的乳酸菌株 （乳桿菌 VN156）。實驗中，乳桿菌 VN156是培養在MRS培養基上生長。培養基的初始 pH值為 5.6。在培養後不同時間測量細菌細胞樣本在 600 nm 的吸光值 (OD)(圖3.1)。OD_{600} 時的數值1相對應於 $2x10^8$ 細胞/mL.。樣品 A0、A2、A3、A5為所收集的上清液樣本，將用於乳酸生產分析。

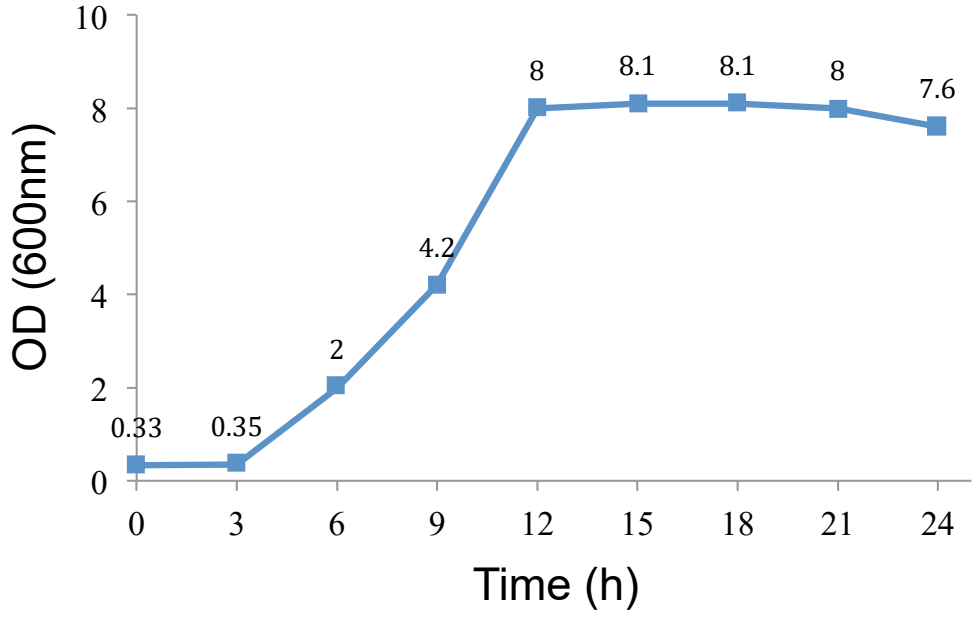


Fig.3.1. Growth curve of Lactobacillus sp. VN156VN156
Lactobacillus sp. VN156的生長曲線

Q.3.1. (3 POINTS分)

Assume that the Fig 3.1 represents the real course of the growth. Calculate the generation time (h) of Lactobacillus sp. VN156 during exponential phase and record the value in the Answer Sheet.
假設圖3.1代表真正的生長過程。計算乳桿菌 VN156在指數成長階段的世代時間(h)，將數值記錄在答案卷上。

Q.3.2. (3 POINTS分)

If 1 mL of the culture at 9 h is diluted into fresh MRS medium, calculate the number of bacterial cells after 6 h of cultivation and record the value in the Answer Sheet.
如果將9 h 培養液1 mL稀釋於新鮮的MRS培養基中，計算6 h後培養的細菌細胞數量，將數值記錄在答案卷上。

Calibration of the pH meter
pH meter 的校正

Use Hanna portable pH meter (Fig 3.2) for measuring the pH
用Hanna攜帶式pH meter (圖3.2) 測量 pH

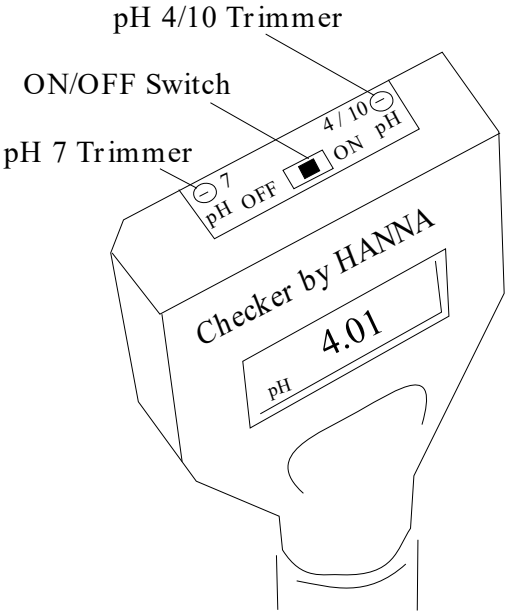


Fig 3.2. Hanna portable pH meter

Calibrate the pH meter according to the following procedure Fig 3.3.
依圖 3.3所描述方式校正pH meter

- Turn the pH meter on by pressing ON/OFF button.
  按 ON/OFF 鍵打開pH meter.
- Remove the protective cap and rinse the tip of the electrode with water, gently wipe with tissue paper.
  取下保護套將電極浸在水中, 以紙巾輕拭.
- Dip the tip of the electrode in pH 7.01 buffer solution. Ensure the electrode tip and junction are fully immersed in the solution (about 2 cm of the tip is in the solution). Allow the reading to stabilize.將電極尖端浸於 pH 7.01緩衝液中. 確保電極尖端和交界處完全浸在溶液（約 2 cm的尖端在溶液中）。等讀取值穩定。
- Use a screwdriver to adjust the pH 7 trimmer until the display reads pH 7.01
  用小螺絲刀調整 pH 7 微調直到讀取 ph 值顯示為 7.01。
- Rinse the pH electrode with water, gently wipe with tissue paper.
  用水沖洗pH 電極，用紙巾輕輕地擦拭。
- Dip the tip of the electrode in pH 4.01 buffer solution. Allow the reading to stabilize.
  將電極尖浸於 pH 4.01緩衝液中. 等讀取值穩定。
- Use the screwdriver to adjust the pH 4 trimmer until the display reads pH 4.01.
  用小螺絲刀調整 pH 4 微調直到讀取 ph 值顯示為 4.01。
- Calibration is completed. 完成校正
- Note: if you switch off the pH meter you should calibrate it again
  注意: 如果你關掉過pH meter 要再使用必須重新校正

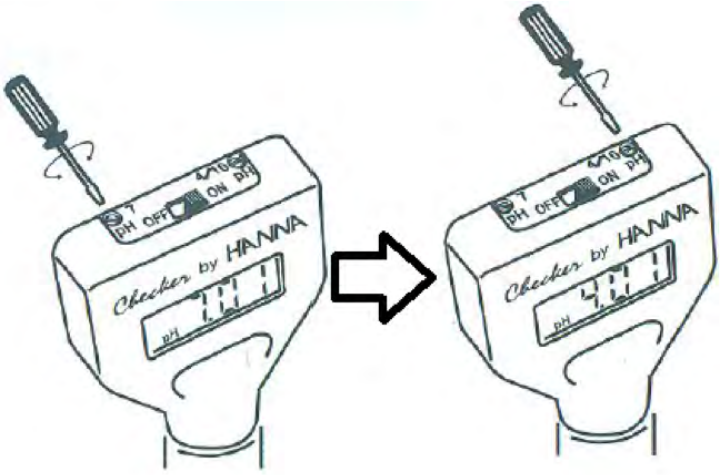


Fig 3.3. pH Calibration

Lactic acid titration 乳酸滴定

Titration is an analytical technique, which allows the quantitative determination of a specific substance (analyte) dissolved in a solution. It is based on a complete chemical reaction between the analyte and a reagent (titrant) of known concentration, which is added to the solution. In this part, titration method will be used to determine lactic acid concentration in the samples by titration with 0.1M sodium hydroxide solution as shown in Fig.3.4.
滴定是一種分析技術，用以定量測定溶在水溶液中的特定物質 （受分析者） 。它是基於受分析者和添加到溶液中已知濃度的試劑 （滴定者）間完全的化學反應。本實驗如圖 3.4 所示，將用滴定法於以0.1 M 氫氧化鈉溶液滴定以確定樣品中的乳酸濃度。

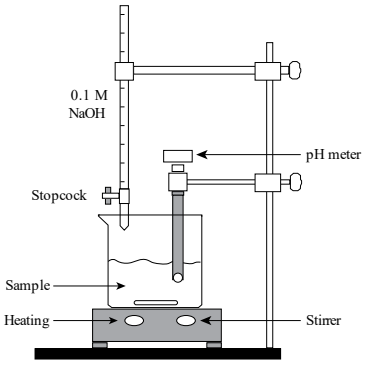


Fig.3.4. Titration setup

Q.3.3. (2 POINTS分)

Calculate the volume of sample (mL) and water (mL) needed to be used according to the table in Q.3.2 and record the values in the table provided in the Answer Sheet.
根據Q.3.2的表計算所需使用之樣本及水的體積 (mL)，將數值記錄在答題卷的表中。

1. Based on your calculation, prepare sample dilutions for each sample in 100 mL beakers. Prepare two duplicates for each sample using 25 mL measuring cylinder for deionized water, micropipette and 10 mL measuring cylinder for samples
根據你的計算，以100 毫升燒杯為每個樣品製備稀釋液。用25 毫升量筒測量去離子的水、微量吸管和10 毫升樣品量筒製備二個樣品的重複。.
2. Carefully place a magnetic stirring bar into the diluted sample solution. Clamp the pH meter and position the pH electrode deep in the solution (about 2 cm of the tip is in the solution) so that the stirring bar will not hit the pH electrode while stirring. Begin stirring slowly and record the starting volume of 0.1M NaOH solution.
將磁性攪拌棒小心放入稀釋的樣品溶液中。夾好 pH 計，注意讓 pH 電極的位置是浸在溶液中（前端約 2 cm浸在溶液中）而攪拌棒不會打到電極。慢慢攪拌並要記錄0.1 M NaOH 溶液起始時的體積。
3. Open the stopcock of the burette to allow the NaOH solution from the burette to slowly run into the sample in the beaker. Stop adding NaOH when the pH of sample changes to neutral (6.95-7.05). Record the final volume of 0.1M NaOH solution.
打開滴管旋塞的閥，讓NaOH 溶液慢慢滴入裝樣品的燒杯裡。當樣品的 pH值變為中性 (6.95-7.05)時，就停止添加氫氧化鈉。記錄 0.1 M NaOH 溶液的最終體積。
4. After each titration, carefully remove the pH electrode from the solution, rinse it with water. Remove the stir bar by using a pair of forceps and rinse it with water.
每次滴定後，從溶液中小心移出pH 的電極並用水沖洗乾淨。使用鑷子移出攪拌棒也用水沖洗乾淨。
5. Repeat steps 2–4 with a new sample.
對每個新樣本重複步驟 2 到4。

Q.3.4. (9 POINTS分)

Record the volume of 0.1 M NaOH used to titrate each sample in the table provided in the Answer Sheet.
在答案卷的表上紀錄滴定每個樣品所使用之0.1 M NaOH的體積

Q.3.5. (10 POINTS分)

Calculate the mean volume of 0.1 M NaOH needed to be used to titrate 30 mL of each stock sample and the concentration of lactic acid in each sample based on the final volume of base titrated. Record all values in the table provided in the Answer Sheet.
計算用於滴定 30 毫升每種樣品所需0.1 M 氫氧化鈉的平均體積，及每個樣本中的乳酸濃度。在答案卷的表中記錄所有的數值。

Note: NaOH (Mw=40) and lactic acid -$C_3H_6O_3$ (Mw=90)

Q.3.6. (3 POINTS分)

Based on Fig 3.1, assuming that the OD_{600} of 1.0 equals to the densitiy of 2×10^8 cells/mL. The concentration of lactic acid will be increased 1g/L if the number of bacterial cells increases 2×10^8 cells/mL. If at 11 hours of cultivation the lactic acid concentration is 6 g/L, calculate the number of bacterial cells (cells/mL) and record the value in the Answer Sheet.
根據圖3.1，假設的OD_{600}時的1.0等於 2×10^8 細胞/ mL的密度。細菌細胞的數量若增加 2×10^8 細胞/ mL，乳酸濃度將增加 1 g/L。若培養 11 h 時乳酸濃度為 6 g/L，計算細菌細胞 (細胞/mL) 的數量並在答案卷上記錄其數值。

記錄在答案卷上

END OF PRACTICAL 3　實作3結束

Country 國家:
Student Code 學生代碼:

27th International Biology Olympiad

July 17-23, 2016
Hanoi, Vietnam

Practical Exam 3 實作測驗 三

BIOCHEMISTRY and 生化與 MICROBIOLOGY 微生物學

ANSWER SHEET 答案卷

Total points: 100 總分: 100分
Duration: 90 minutes 時間: 90分鐘

Q.1.1 (6 POINTS)
問題.1.1(6分)

	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
Sample	Protein Marker	NO_IPTG	IPTG	Pellet	Super	Puri-P
Stock (µl)	5			14.9	14.9	15
H_2O (µl)	5			12.5	12.5	12.5
2X SDS-PAGE buffer	10			12.6	12.6	12.5
Total volume (µl)	20	40	40	40	40	40

Q.1.2. SDS-PAGE RESULT (10 POINTS)
問題.1.2.SDS-PAGE 結果 (10 分)

Attach your picture here

Q.1.3 (4 POINTS)
問題.1.3(4 分)

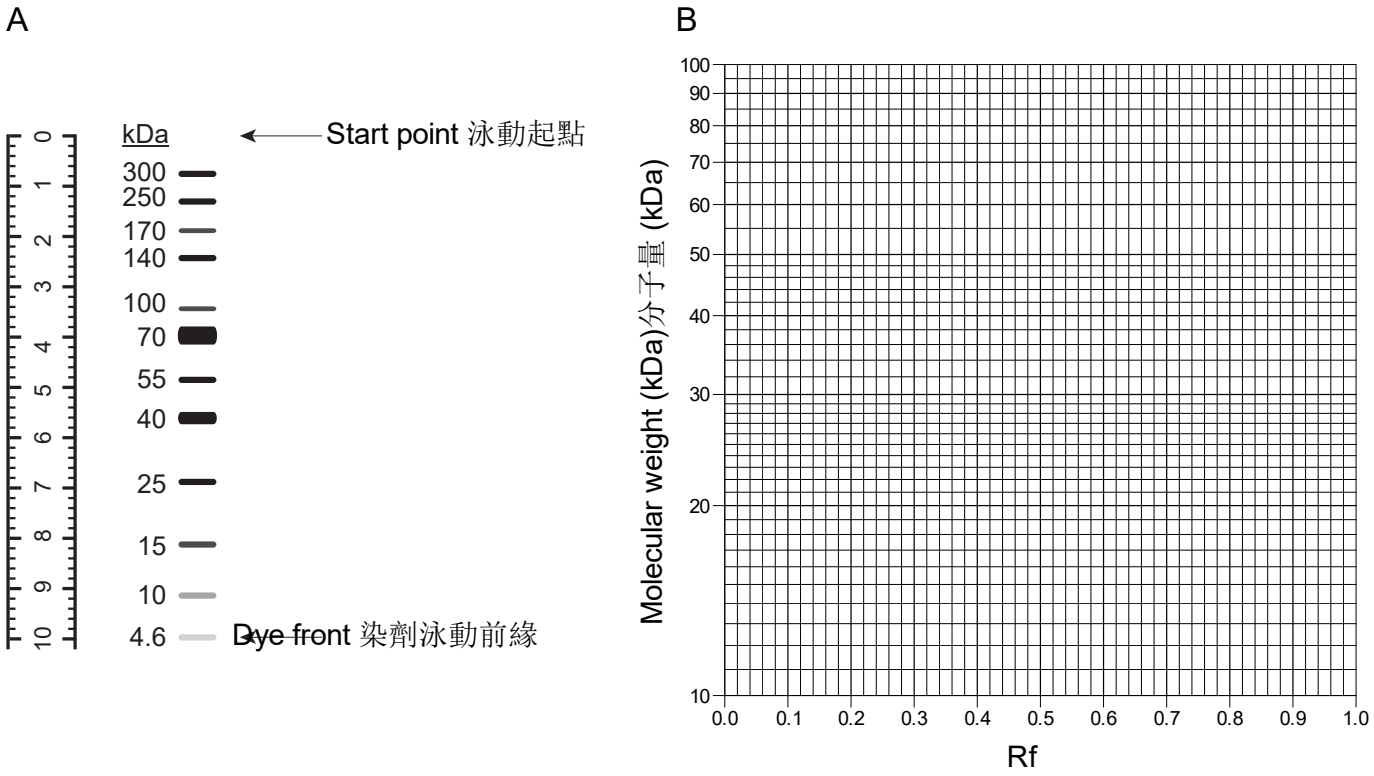


Fig.1.4. Protein markers (A) and graph paper (B)
圖.1.4.蛋白質分子量指標(A)和方格圖紙(B)

Q.1.4. (4 POINTS)
問題.1.4.(4 分)

Protein 蛋白質	Size (kDa) 分子量(kDa)
H	
B	

Q.1.5 (4 POINTS)
問題.1.5(4 分)

	True 正確	False 錯誤
A		
B		
C		
D		

Q.1.6. (4 POINTS)
問題1.6.(4 分)

	True 正確	False 錯誤
A		
B		
C		
D		

Q.1.7. (4 POINTS)
問題.1.7.(4 分)

Sample 樣本	Size (kDa) 分子量(kDa)
The peak observed in sample 1 樣本1 層析結果的波峰位置觀察	
The peak observed in sample 2 樣本 2 層析結果的波峰位置觀察	

Q.1.8. (4 POINTS)
問題1.8(4分)

	True 正確	False 錯誤
A		
B		
C		
D		

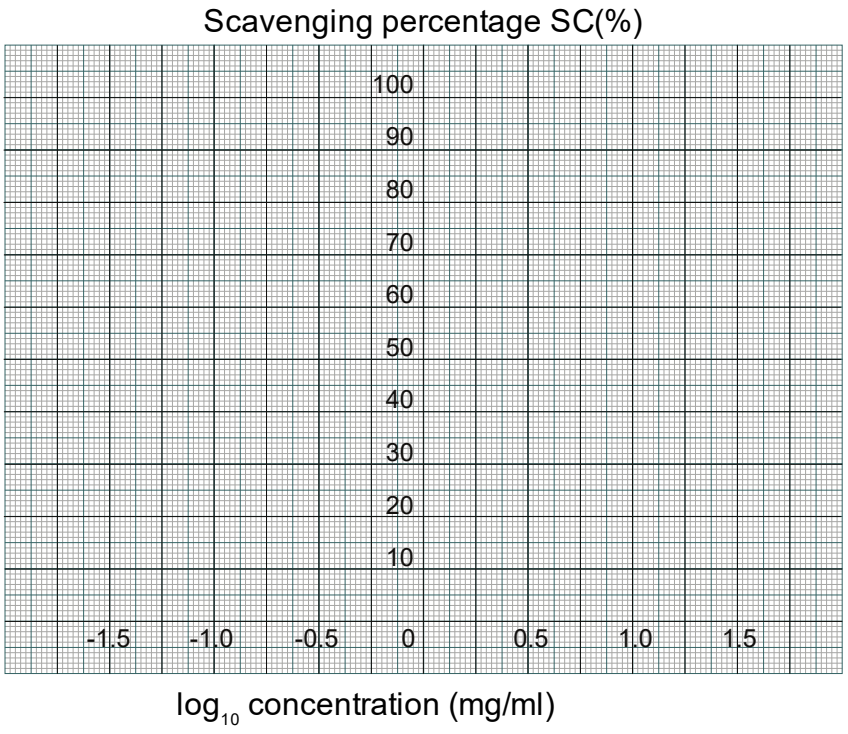
Q.2.1 (4 POINTS分)

Diluted solution 稀釋溶液	AA1	AA2	AA3	AA4	CC1	CC2	CC3	CC4
Volume (µl) of solution for dilution 稀釋溶液體積								
Volume of H_2O 水體積 (µl)								
Concentration 濃度(mg/mL)				0.025				0.625

Q.2.2 (5 POINTS分)

Solution溶液	Control	AA1	AA2	AA3	AA4	CC1	CC2	CC3	CC4
Log_{10} concentration 濃度(mg/mL)									
Mean absorbance平均吸光值									
SC%									

Q.2.3. (5 POINTS分)



Q.2.4. (5 POINTS分)

	Ascorbic acid 抗壞血酸	Coffee extract 咖啡萃取物
SC_{50}		

Q.2.5. (3 POINTS分)

	>		>	

Q.2.6. (4 POINTS分)

	True對	False錯
A		
B		
C		
D		

Q2.7. (4 POINTS分)

	True對	False錯
A		
B		
C		
D		

Q.3.1. (3 POINTS分)

Generation time　世代時間	

Q.3.2. (3 POINTS分)

Number of bacterial cells 細菌細胞數量	

Q.3.3. (2 POINTS分)

Cultivation time培養時間 (h)	Sample樣品	Dilution factor 稀釋倍數	Sample volume 樣本體積(mL)	Deionized water volume 去離子水體積(mL)	Total volume 總體積(mL)
0	A0	2			30
6	A2	5			30
9	A3	10			30
15	A5	20			30

Q.3.4. (9 POINTS分)

培養時間 Cultivation time (h)	Diluted sample稀釋的樣品	Volume of 0.1 M NaOH titrated (First replicate 第一重複,mL)滴定用的0.1 M NaOH體積	Volume of 0.1 M NaOH titrated (Second replicate第二重複,mL)滴定用的0.1 M NaOH體積	Average volume of 0.1 M NaOH titrated (mL)滴定用的0.1 M NaOH平均體積
0	A0			
6	A2			
9	A3			
15	A5			

Q.3.5. (10 POINTS分)

Cultivation time 培養時間(h)	Sample 樣品	Volume of 0.1M NaOH (mL) used to titrate 30 mL of stock sample滴定 30 毫升樣品所需0.1 M 氫氧化鈉的平均體積(mL)	Lactic acid production 產生之乳酸(g/L)
0	A0		
6	A2		
9	A3		
15	A5		

Q.3.6. (3 POINTS分)

細菌数目 (細胞/mL)	