27th International Biology Olympiad

July 17-23, 2016
Hanoi, Vietnam

Practical Exam 4　實作測驗四

MOLECULAR BIOLOGY　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 exam, you are going to perform a combined PCR-RFLP experiment for two purposes simultaneously:
本測驗中你要進行一個結合聚合酶連鎖反應-限制酵素切割片段長度多型性(PCR-RFLP)的實驗，同時達成兩個目的:

1. **Genotyping drug metabolising enzyme (NAT2) to determine relevant oral drug dosage in treatment of tuberculosis (TB) patients.**
   藥物代謝酵素**(NAT2)**之基因型鑑定以決定治療肺結核**(TB)**患者之口服劑量
2. **Forensic identification of unidentified biopsy specimens**
   未確認生物檢體之法醫鑑定

The experiment consists of five tasks:

- Task 1: Design of RFLP experiment (17 points)
  任務 1: 設計RFLP實驗 (17分)
- Task 2: Performance of RFLP experiment (44 points)
  任務2: 執行RFLP實驗 (44分)
  (*Notice:* Electrophoresis must start not later than **75 minutes** after the exam begins. After this, you will NOT be allowed to run the gel).
  注意
  本測驗開始後的75分鐘內必須開始電泳，逾時則不准跑膠。
- Task 3: Forensic identification of unidentified biopsy specimens (9 points)
  任務3: 未知生物檢體之法醫鑑定(9分)
- Task 4: Interpretation of patients' genotypes (12 points)
  任務4: 病人基因型判讀 (12分)
- Task 5: Determination of drug dosage relevant to patients' genotypes (18 points)
  任務5: 決定給予不同基因型病人之藥物治療劑量(18分)

Please take note of the following:
請注意下列事項:

- Please remember to write your Country and Student code in the given box
  請記得用鉛筆在每張答案卷的指定方格中填上你的國家代碼和學生編號
- Write your answers in the separate **Answer Sheets** (using pencil and eraser). Only answers given in the **Answer Sheets will be evaluated**.
  請用鉛筆將你的答案清楚寫在答案卷上,只有寫在答案卷上的答案會被評分
- Make sure that you have received all the materials and equipment listed at the beginning of the exam. If any of these items are missing, please raise the **Red card** immediately to notice the lab assistants.
  實驗開始前請先依據器材單確認你具有所有的材料及儀器,如有任何缺少請即舉起你的紅牌，請助教協助補充

- During the experiment, ensure to handle the equipment properly. Any spilled chemicals or broken equipment will **Not** be replenished. However, if any equipment appears to malfunction, please raise the **Red card**. A Lab assistant will come to help and if necessary replace the equipment .
  實驗中請注意小心操作儀器,任何打翻化學藥品及毀損儀器將不會再補充,如儀器有功能異常請舉紅牌，請助教協助恢復或更換儀器
- Stop answering and put down your pen immediately when the bell rings at the end of the exam. Enclose the **Answer Sheets**, **Question papers**, and **Data printout** in the provided envelope.
  考試終止鈴聲響時請停止作答,放下筆並將答案卷**,**問題卷及數據列印卷放入大會提供的信封袋中
- No paper or materials should be taken out of the laboratory.
  請勿將紙張及實驗材料攜出實驗室

祝好運!

實驗材料與儀器

Materials and equipment 實驗材料與儀器	**Quantity** 數量
FlashGel™ horizontal electrophoresis chamber with lighting switch 具光源開關的FlashGel™水平電泳及膠片觀察槽	1 piece 1個
FlashGel™ (precast) agarose gel cassette (in sealed bag) 在封袋中已注膠完成之FlashGel™洋菜膠	1 piece 1個
Electrophoresis power supply (one for 2 students; operated by lab assistants) 電泳電源供應器(每2位學生一台,由實驗助教操作)	1 piece 1臺
Water-bath 37ºC (one for 4 students; located behind your seat) 37ºC水浴槽(在你座位後面,4位學生共用一台)	1 set 1個
Heat block 80ºC (one for 4 students; located behind your seat) 80ºC乾浴槽(在你座位後面,每4位學生1台)	1 set 1個
Micro-centrifuge (spin-down) with adapters for 0.2 / 1.5 mL tubes 微量離心機(具有0.2/1.5 ml離心管轉用器)	1 set 1臺
Micropipette P200 200 ul 微量吸管P200	1 piece 1支
Micropipette P20 20 ul 微量吸管P20	1 piece 1支
Sterile micropipette tips in box for p20 供 p20 微量吸管用之無菌微量吸管尖	1 box 1盒
Sterile micropipette tips in box for P200 供 p200 微量吸管用之無菌微量吸管尖	1 box 1盒
Ice box filled with flaked ice (with cover) 裝有碎冰的冰盒	1 box 一盒
1.5 mL microfuge tube rack 1.5 ml微量離心管架	1 piece 1個
0.2 mL microfuge tube rack 0.2 ml微量離心管架	1 piece 1個
1.5 mL microtubes 1.5 ml 微量離心管	5 pieces 5管
0.2 mL microtubes (PCR tubes) 0.2 ml微量離心管(PCR管)	15 pieces 15管
Stopwatch 碼錶	1 piece 1個
Foam floating rack (15 holes for 0.2 mL microtubes) 浮水海綿試管架(15孔, 0.2ml微量離心管用)	1 piece 1個
Green card to signal assistant(s) for proceeding experiment 綠卡 (指示請助教進行後續實驗用)	1 piece 1張
Red card to signal assistant(s) for technical problem(s)/supports 紅卡 (請助教協助用)	1 piece 1張
A tip disposal container (plastic beaker with lid) 具蓋子的吸管丟棄筒	1 piece 1個
Polygloves (disposable gloves) 丟棄式實驗手套	3 pairs 3雙
Twin marker pen (permanent ink) 永久墨水實驗標示筆	1 piece 1支
Student code sticker (to attach to your worked-out image) 學生號碼標簽黏貼紙(用以張貼結果數據用)	1 piece 1支
Kimwipe paper for blotting excess liquid on precast gel cassette	1 box

擦拭紙(用以吸取電泳膠組多餘的液體)	1盒
Tissue (Pussy®) paper for cleansing bench/equipment (if needed) 衛生紙(清整實驗桌面用)	1 box 1盒
Safety goggle 安全護眼鏡	1 piece 1只
Squirt bottle containing deionized water (500 mL) 裝去離子水的洗瓶	1 bottle 1只
Scissor (to unpack the bag containing precast gel cassette) 剪刀 (用以剪開預注膠體包裝袋)	1 piece 1把

Other tools, including handy calculator, pencil (2B Type), eraser (for pencil) and ruler you are provided for commonly using in all the labs.
會提供其他常用工具,包含計算機,2B鉛筆,橡皮擦及尺等，你也可用於其他場的實作測驗

Reagents	**Quantity**
PCR products of *NAT2* gene derived from three patients (green caps labeled P1, P2 and P3) 三位病人之NAT2基因PCR反應產物(綠色蓋上分別標示P1, P2及P3)	3 tubes x 10 µL 三管,每管10 uL
PCR products of *NAT2* gene derived from unidentified biopsy specimens (red caps labeled X, Y and Z) 三個未知生物檢體之NAT2基因PCR反應產物(紅色蓋上分別標示X, Y及Z)	3 tubes x 10 µL 三管,每管10 uL
Restriction enzyme *Kpn*I, labeled RE1 (green label) 限制酶*Kpn* I, 標示RE1(綠色標籤)	1 tube x 10 µL 1管, 10 µL
Restriction enzyme *Bam*HI, labeled RE2 (blue label) 限制酶*Bam* HI, 標示RE2(藍色標籤)	1 tube x 10 µL 1管, 10 µL
10x Restriction buffer, labeled BF (purple tube) 10x限制酶緩衝液, 標示BF(紫色標籤)	1 tube x 50 µL 1管, 50 µL
MiliQ water tube, labeled W (white label on blue tube) 裝MiliQ 超純水的管子, 標示W (白標籤標示之藍色管子)	1 tube x 200 µL 1管 200 uL
DNA staining dye, labeled D (red label on red tube) DNA染劑, 標示D (紅標籤標示之紅色管)	1 tube x 50 µL 1管, 50 µL
100 bp DNA ladder, labeled M (orange label on yellow tube) 100 bp DNA尺標,標示 M (橘色標籤之黃色管)	1 tube x 10 µL 1管, 10 µL

TASK 1. DESIGN OF PCR-RFLP EXPERIMENT (17 POINTS)
任務1. PCR-RFLP實驗設計(17分)

Introduction
內容介紹

Isoniazid (INH) is a pivotal agent in first-line anti-tuberculosis (TB) treatment.
Despite the rather successful therapeutic effects of this regimen, there are still treatment failures (ineffective treatments) and unmanageable side effects (most commonly liver injury and occasionally mortality).
Isoniazid (INH)是對抗肺結核病之第一線用藥,
雖然此用藥的治療效果很成功,但仍會有治療失敗，以及無法控制的副作用(常造成病人肝臟受損, 有時會造成病人致死)

INH acetylation was found to be the major contributor to drug-induced hepatotoxicity.
INH乙醯化被發現是造成此藥誘導肝毒性之主要原因

Figure 1 presents the major pathway for INH acetylation catalysed by non-inducible hepatic enzyme arylamine N-acetyltransferase type 2 (NAT2).
圖一顯示肝臟非誘導型的NAT2基因編碼酵素為催化INH乙醯化之主要路徑

The rate of acetylation is constant in an individual but varies between patients
個體本身之乙醯化速率雖為恆定,但在病患個體間的乙醯化速率卻有差異性

The human population can be divided into three different phenotypic groups according to acetylation rate: slow, intermediate and rapid acetylators.
人類族群可依每人的乙醯化速率而分屬下列三種不同表現型:慢乙醯化能力者,中乙醯化能力者, 快乙醯化能力者

It is well known that INH-induced hepatotoxicity develops more frequently in NAT2 slow-acetylators.
已知INH較常在NAT2慢乙醯化能力者誘導出肝毒性

In contrast, treatment failure is likely to occur in rapid-acetylators.
但相反的,治療失敗常發生在具NAT2快乙醯化能力的病患

Most commonly, rapid-acetylators are those whose genotypes are homozygous for the wild-type SNP allele at all the three positions, thus NAT2*4 (C481, G590, G857)
通常快乙醯化能力者具有野生型同型合子基因,其3個不同位點都具野生型SNP,亦即NAT2*4 (C481, G590, G857)

Intermediate-acetylators are heterozygous for a mutant SNP allele at a single locus, ie one out of the 3 loci NAT2*5 (C481T), NAT2*6 (C590A) or NAT2*7 (G857A)
而中乙醯化能力者為具有單一個突變型SNP等位基因之異型合子之個體。突變型SNP等位基因為NAT2*5(C481T), NAT2*6(C590A) 或 NAT2*7(G857A)

(**Figures 2a** and **2b**). Slow-acetylators are those who have more than one mutant alleles (either two alleles at one position or multiple positions)
(圖**2a**及**2b)**慢乙醯化能力者為具有多於一個突變等位基因者(此2個突變SNP位在同一位置,或位在多個位置上)

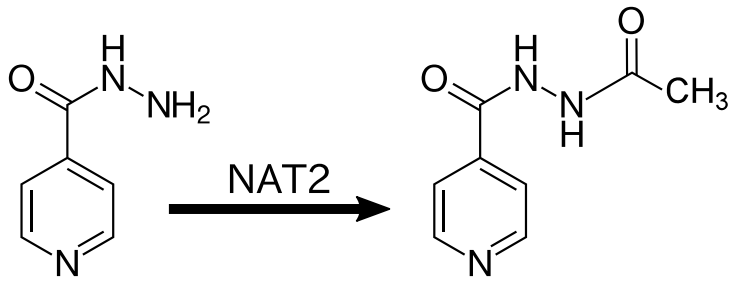


Figure 1. Metabolism of isoniazide catalysed by NAT2 (N-acetyltransferase)
圖1. NAT2(N-乙醯轉移酶)催化代謝isoniazide路徑

The NAT2 genotype can be determined by using an allele-specific polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay.
個體之NAT2基因型可以用單一等位基因特異性聚合酶反應-限制酶切割片段長度多型性分析方法進行鑑定

Analysis of INH concentrations in the blood of patients of different NAT2 genotypes receiving the same doses of INH revealed that the serum concentration of INH was 2-to 7-fold higher among slow-acetylators compared to rapid- and intermediate-acetylators.
分析病人血中INH濃度顯示不同NAT2基因型之病人服用相同劑量之INH後,血清中的濃度在慢乙醯化能力者比快乙醯化能力者及中乙醯化能力者高出2-7倍

Thus, genotyping NAT2 enables personalization of INH doses.
因此NAT2基因型鑑定有助於決定患者個人化INH治療劑量

In this experiment, you will receive PCR products of *NAT2* gene derived from total genomic DNA of three TB patients P1, P2 and P3, and PCR products of 3 unidentified biopsy specimens from these 3 patients labelled X, Y and Z.
本實驗中你會收到由P1, P2及P3三位結核病患者之基因體DNA進行PCR增幅之*NAT2*基因PCR反應產物,及分別標示為X, Y 及Z之3管由這三位TB患者採集的未確認生物檢體之*NAT2*基因PCR反應產物

You are to design and perform a combined PCR-RFLP experiment to determine *NAT2* genotype for each patient and forensically identify their biopsy specimens.
請你設計並執行PCR-RFLP實驗用以鑑定每一病人及其未被確認檢體之*NAT2*基因型

To determine the genotype, appropriate restriction enzymes (RE) are used on the PCR products. With the data obtained, identify biopsies X, Y and Z.
為了鑑定基因型請選用適合之限制酶切割PCR反應產物,並利用所獲得的結果資訊以確認未知生物檢體X, Y及 Z之基因型

Finally determine the appropriate dose of INH for patients P1, P2 and P3.
最後並請決定適合病患P1, P2及P3的治療劑量

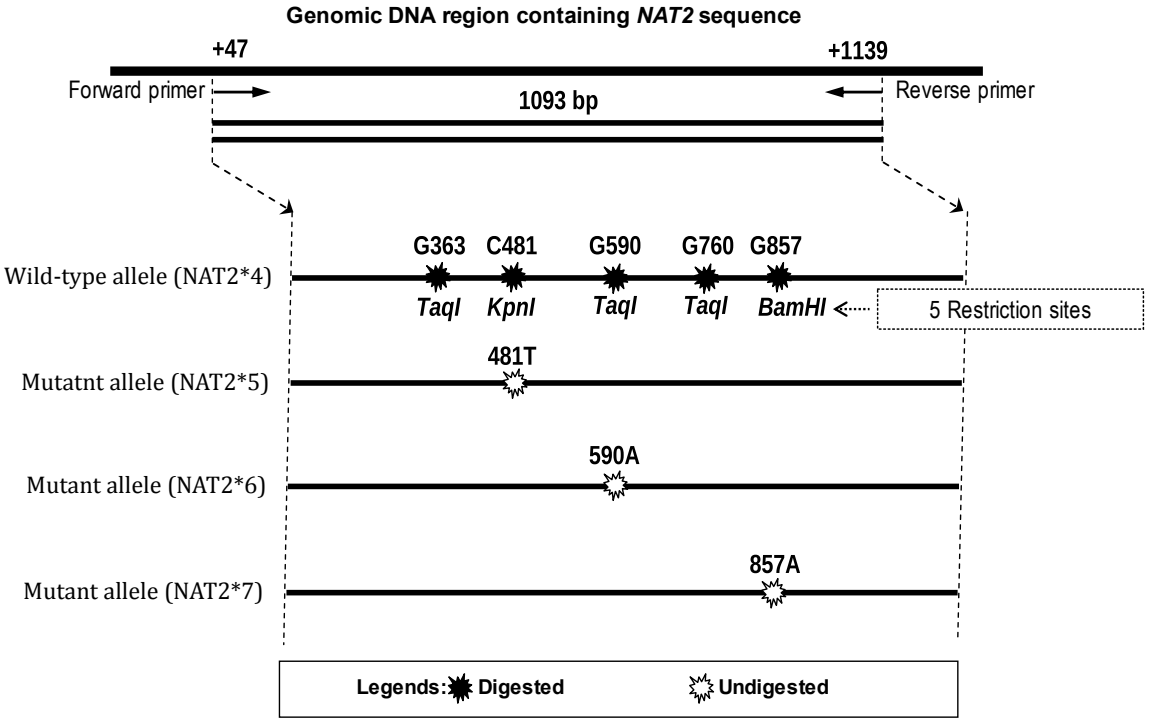


Figure 2(a). Restriction sites of the three restriction enzymes (REs) *Kpn*I, *Taq*I, and *Bam*HI in the gene coding for N-acetyl transferase type 2 (NAT2). These REs are used to generate PCR-RFLP fingerprints for detecting mutant alleles NAT2*5, NAT2*6 and NAT2*7 as distinguished to the wild-type allele NAT2*4. Forward and reverse PCR primers anneal correspondingly to the +47 and +1139 from the start codon of *NAT2* gene.

圖**2(a)**. NAT2基因DNA上之限制酶(REs) *Kpn* I, *Tag* I及*Bam* H1 切割位點, 這些REs 可用以獲得PCR-RFLP指紋用以檢定突變等位基因 NAT2*5, NAT2*6及NAT2*7,因其可與野生型NAT2*4等位基因產生的指紋有區別, 正向及反向PCR引子會分別與距*NAT2*基因起始密碼子47鹼基位置(+47)及1139鹼基位置(+1139)位置煉合

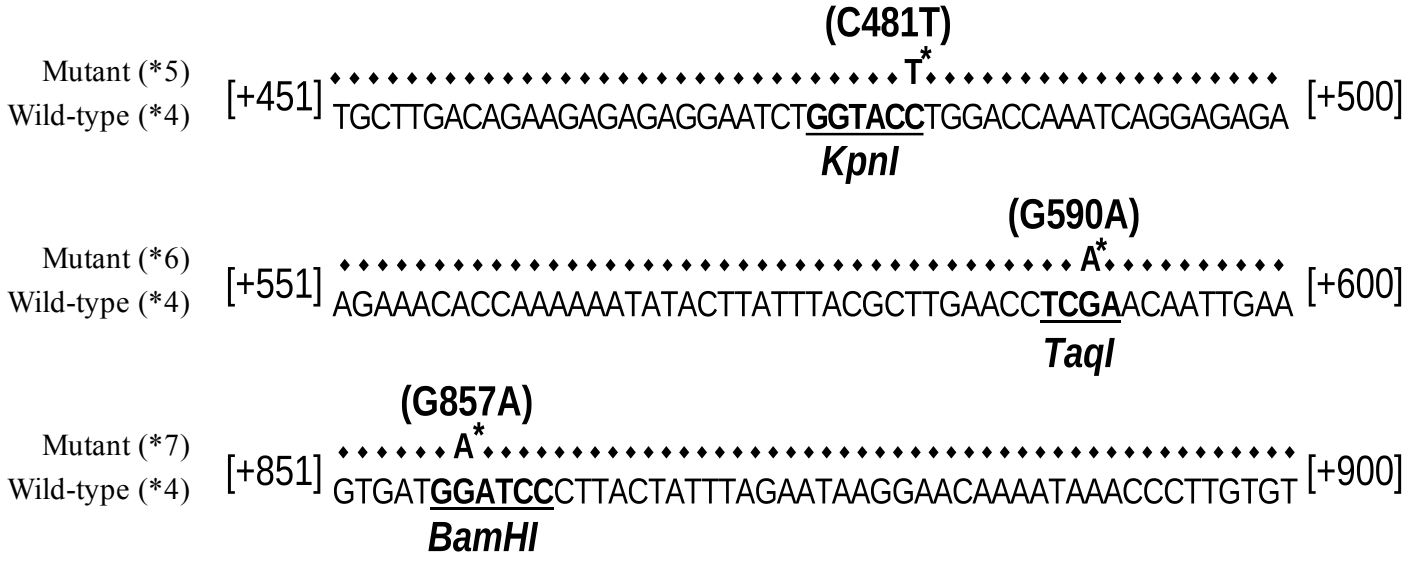


Figure 2(b). Truncated sequence of PCR products of wild-type and mutant NAT2 alleles. Numbers in brackets at the beginning and the end of each row indicate the first and the last base of the presented sequence of the wild-type allele (NAT2*4; represented in A/T/G/C) and of the corresponding mutant alleles (NAT2*5, NAT2*6, and NAT2*7) where dot marks (♦) reveal the nucleotides identical to the wild-type allele. A*/T* presents the SNP mutants.

圖**2(b)**. NAT2等位基因PCR產物之野生型及突變型的部分序列。
每行前後大括號中的數字標示出以NAT2野生型(NAT2*4)等位基因為基準時，此部分序列之開始及最後鹼基的編碼位置，序列以A/T/G/C呈現如圖中所示。而相對應之突變型等位基因(NAT2*5, NAT2*6及NAT2*7)，其序列中的 (♦)表示其鹼基與野生型等位基因的鹼基相同,而A*或T*則為突變型SNPs之所在位置。

問題**1.1 (12**分**)**

Complete the expected RFLP patterns in the figure provided on the **Answer Sheet** by drawing in pencil the expected bands of completely RE digested PCR products of the four *NAT2* alleles: NAT2*4 (wild-type), NAT2*5 (481T), NAT2*6 (590A) and NAT2*7 (857A). Examples are already given for heterozygotes.

請用鉛筆在答案卷的圖形中畫出四種*NAT2*等位基因: NAT2*4 (wild-type), NAT2*5 (481T), NAT2*6 (590A) and NAT2*7 (857A)的PCR產物經限制酶完全切割後，預期的片段條帶之圖譜，圖中以異型合子的結果為例。

For this task, you are required to perform RFLP reactions in a total of 12 tubes for genotyping the wild-type and the two alleles NAT2*5 (labelled with a) and NAT2*7 (labelled with b) for each patient (P1, P2 and P3) and their biopsy specimens (X, Y and Z). Always use 7.0 μg DNA, restriction buffer and where appropriate use 1.0 μL RE, per 0.2 mL microtube (PCR tube).
在本任務中請你執行12管PCR-RFLP實驗,進行針對每個病人(P1, P2 and P3)及其未知生物檢體之野生型及2個突變等位基因NAT2*5(標示為a) 及 NAT2*7(標示為b)之基因型鑑定, 在每個0.2 mL 管中的反應請都使用7.0 μg DNA, 限制酶緩衝液及1.0 μL 適當的限制酶進行實驗。

Q1.2 (5 POINTS)
問題1.2 (5分)

Design your restriction digests or *NAT2* genotyping of Patients (P1 – P3) and specimens (X - Y) in a total volume of 10 μL by completing the table provided in the **Answer Sheet**. 請以10 μL 的總反應體積設計你的限制酶切割實驗或病患(P1-P3)及檢體 (X - Y)的*NAT2*基因型鑑定實驗,並在答案卷中所提供之表格中填入反應物之體積量

TASK 2. PERFORMANCE OF RFLP EXPERIMENT (44 POINTS)
實驗二. 執行RFLP實驗 (44分)

Notes: 注意

1. For electrophoresis, you are handling two parts of the FlashGel® System, 12+1-well Cassette and Dock (**Figure 3**), while the Power Supply and Camera are operated by Lab assistants. For best results, flood the wells with deionized water prior to sample loading. To observe the bands, turn on the light (using the knob on the Dock), and wear the safety goggles.

   電泳部分，你要操作FlashGel®系統中的二部分為: 12+1樣品孔的膠組和底座 (圖3)。電源供應器和相機則由實驗助理操作。為求最佳結果，先在樣品孔中注滿去離子水，再小心注入樣品。要觀看膠體中DNA條帶時，可戴上安全護目鏡並打開底座上的燈光開關。

2. You may request for a second Cassette (precast gel) but there will be a penalty of 20 points.
   你可以再要求一個新的預注膠體組，但會被扣20分。
3. Spin down all reagents in microtubes before directly pipetting (be sure to balance the micro-centrifuge by placing the microtubes opposite each other. If there is only one tube, balance with an empty tube).
   先將所有離心管中的試劑離心下來，再開始吸取樣品。(確認離心時要平衡放置離心管，若只有一管時，用一個空離心管平衡)。

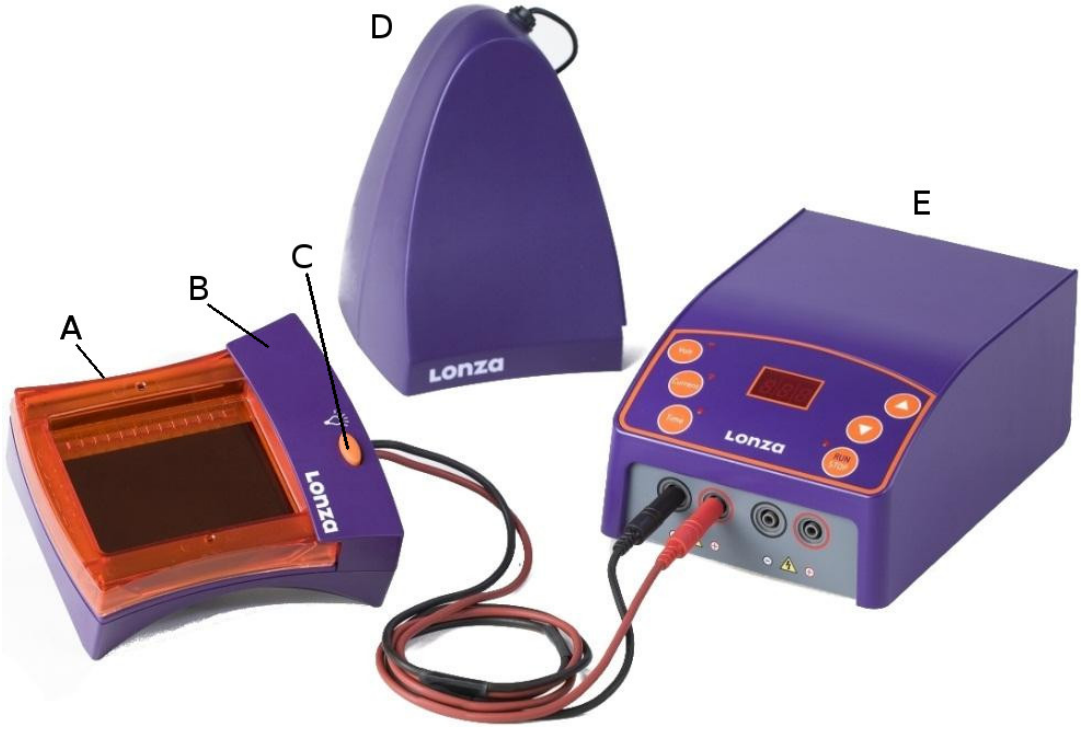


Figure 3. FlashGel® Horizontal Electrophoretic System. **(A)** Cassette. **(B)** Dock. **(C)** Knob for lighting. **(D)** Camera. **(E)** Power supply.
圖3. FlashGel® 水平電泳系統(A)膠體組 (B)底座 (C)光源開關鈕 (D) 相機 (E)電源供應器

This protocol consists of two stages: PCR-RFLP digestion and electrophoresis:
本操作程序包含二階段: PCR-RFLP切割和電泳

Step 1 (*Preparation of microtubes for RE's digestion reaction*)**:** Label 12 microtubes with the fine tipped marker pen as P1a, P1b, P2a, P2b, P3a, P3b, Xa, Xb, Ya, Yb, Za and Zb to correspond to PCR products of genomic DNA of the three patients (P1, P2 and P3) and the three biopsy specimens (X, Y and Z), digested either with *Kpn*I or *Bam*HI.
步驟1 (準備限制酶切割實驗用的離心管): 用細馬克筆標記12個微量離心管分別為P1a, P1b, P2a, P2b, P3a, P3b, Xa, Xb, Ya, Yb, Za 和 Zb，以對應三位病人(P1, P2 和 P3)及三個生物檢體 (X, Y 和 Z)，分別以*Kpn*I 或 *Bam*HI 切割。

Step 2 (*Preparation of the restriction digestion mixtures*): According to your setting-up of restriction digestion reactions (**Tables 1.2** in your **Answer Sheet)**, prepare the restriction digestion mixtures relevant to each microtube you labelled in Step 1. Gently mix the reagents by pipetting them up and down in each tube or finger-tapping the base of the microtubes. Do not contaminate one sample with another when preparing the mixture (use a new pipette tip for each operation). Spin down the mixture in the micro-centrifuge by using appropriate adapters (please balance the tubes before the centrifugation). During preparation and after spinning, always keep the tubes on ice.
步驟2 (準備限制酶切割試劑混合液): 按照你在答案卷中表1.2所設計之實驗反應組別中對應之限制酶切割的規劃，準備對應步驟1中標示離心管的限制酶切割反應試劑混合液。輕輕地以微量吸管吸排或以手指輕彈管壁使混合，每次使用新吸管尖，避免交叉汙染。以微量離心機將混合液離心至底部(使用適宜的轉接套管，並注意平衡)，製備過程中，要隨時將離心管保存於冰上。

Step 3 (*Incubation of digestion reaction and preparation of precast gel*)**.** After all the tubes have been prepared, remove them from ice and place them into your color-coded foam floating rack and incubate for 5 minutes at 37ºC in the water bath assigned for you (located behind your seat). Make sure to retrieve your own samples after 5 minutes of incubation.
步驟3 (進行限制酶切割反應及準備電泳預注膠體)。待所有反應離心管配製完成後，將離心管放在有顏色標記的浮盤上，在指定的37ºC水浴槽中(在你的座位後方)反應5分鐘。勿忘5分鐘後取回你的樣品。

During 37ºC incubation, you can prepare the Cassette (precast gel) as you were instructed when visiting the Lab the day before, with the steps as follows:
在37ºC反應時，你可以準備電泳膠體組(已預注好)，如同你在前一天參觀實驗室時所學的步驟如下:

1. Use scissor to cut off a side of the bag and carefully take out the Cassette.
   用剪刀剪開袋子的一邊，小心拿出膠組
2. Remove white seals from the Cassette (but do not remove the clear side vent seals).
   移除白色封片，勿移除透明封片
3. Use a squirt bottle to flood the sample wells with deionized water (please be sure to flush all the wells), then tilt the Cassette to drain excess liquid, blot off with Kimwipe paper (do not blot wells directly).
   用洗瓶灌注去離子水於樣品孔，確認所有孔都加水沖洗，然後倒出多餘液體，並以Kimwipe擦拭紙間接將水吸乾。但切勿直接用擦拭紙吸取孔內液體。
4. Insert Cassette into Dock (raise your Red Card if you need assistance) and now your gel cassette is ready for sample loading.
   將膠體組插入底座(若需要協助，請舉起你的紅卡)，此時你的膠體組已可注入樣品。

Step 4 (*Stopping digestion reaction by deactivating REs*): When the 5 minutes of restriction digestion duration is up, retrieve your own tubes and move them into a nearby 80^{o}C heat block (*use **tissue paper** to blot excess liquids from outside of the microtubes if necessary*) and incubate for another 5 minutes.
步驟4 (去活化限制酶以停止切割反應): 當5分鐘的限制酶切割反應時間終了時，取回你的樣品，放入 80ºC的乾熱槽(必要時，用面紙擦去管壁外多餘的水)，留置作用5分鐘。

Step 5 (*Staining DNA/PCR-RFLP products*): After 80^{o}C incubation period, spin down the tubes for cooling off and collecting all reagents to the base of the tubes. Add 2.5 μL of DNA staining dye solution (labeled D) into each microtube. Mix them well, then spin down any residual liquid using micro-centrifuge again.
步驟5 (DNA/PCR-RFLP反應產物之染色): 80ºC處理後，將離心管離心以降溫並讓所有樣品降至管底。分別在每一樣品管中加入2.5 μL的DNA染劑(標示D)。混合均勻再離心收集所有殘附管壁之反應液體。

Step 6. (*Loading samples for electrophoresis*): Load 5 μL of each of the 12 samples (P1a to Zb) and 100 bp ladder solution (labelled M) into the wells (*Notice: do not exceed 5 μL per lane as it is the maximum limit of the well volume*). Make sure you position the pipette tips carefully on top of the wells and gently load the mixtures into the wells without spilling them. Add your samples according to the following scheme of lanes. (from the left side and the wells are away from you).
步驟6. (電泳膠體之樣品注膠): 分別自12個樣品及100 bp梯度尺標(標示M)管中取出5 μL 注入到膠組之樣品孔中(注意: 每一樣品孔的最大容積為5 μL ，不要過量)。小心將微量吸管尖置於孔的正上方，輕巧地將樣品注入樣品孔中，不要溢出。請面對膠體使樣品孔遠離你,並由左邊樣品孔開始依續注入各樣品。

P1a	P1b	P2a	P2b	P3a	P3b	M	Xa	Xb	Ya	Yb	Za	Zb

Step 7 (*Running electrophoresis*): When loading is finished, raise your GREEN CARD. The lab assistant will start the electrophoresis run. The voltage of the power supply should be set up at 200V. After about 7 or 8 minutes (when the fastest band of the ladder M has migrated beyond two third of the gel), raise your GREEN CARD again to notify the assistant to disconnect (turn off) the power supply.
步驟7 (電泳): 完成樣品注膠後，舉起你的綠卡，實驗室助理會來啟動膠體電泳，電源供應器的電壓應設定在200 V，大約7到8分鐘後(當 DNA梯度尺標M的最快條帶超過膠體2/3位置時)。再舉起你的綠卡通知助理來關掉電源供應器。

Step 8 (*Documenting your gel*)**.** The assistant will remove the Dock with Cassette from the power supply. Plug in the Dock to electric socket and turn on the light. Observe the gel (with the safety goggles) and draw in pencil the bands of each lane you observed into **Figure Q.2.1** in the **Answer Sheet** (Notice the scale of the preprinted molecular ladder (M), and any lanes or bands drawn but not matching the gel photograph in next step will not be scored).
步驟8 (記錄你的膠體電泳結果): 助理會將膠體組及底座移離開電源供應器並將底座接到另一電源接頭，並打開光源，請你戴上安全護目鏡並觀察電泳結果，在答案卷的圖Q.2.1上，用鉛筆畫出每丨樣品的電泳條帶(注意圖上的梯度尺標(M)比例，若任丨畫出的樣品行或條帶，與下丨步驟的電泳照片不符時，將不予計分)

Step 9 (*Photographing your gel*). After finishing the drawing, label the sticker with your **Student ID** and affix onto the frame (the red part) of your gel cassette. Raise your GREEN CARD to hand over your whole gel Dock with Cassette to a Lab assistant. The gel will be photographed and its image will be attached onto **Figure Q.2.2** in your **Answer Sheet** by the assistants afterwards (Notice lanes appearing in a wrong position as compared to those described in Step 6 will not be scored, but they can be used in solving next questions of this exam).
步驟9 (電泳膠體之照像): 完成畫圖後，在貼紙上寫上你的**Student ID**，貼在膠組的邊框上(紅色部分)。舉起你的綠卡，將整個膠組連同底座一起交給助理。助理在試後會將電泳膠照像，並將照片貼在你的答案卷圖Q.2.2上(注意，如果樣品行和步驟6指示的順序不符，將不予計分，但可用於解答下列問題)。

Q.2.1. DRAWING OF GEL (18 POINTS)

Q.2.2 PHOTO (26 POINTS)
Q.2.2照片 (26分)

TASK 3: FORENSIC IDENTIFICATION OF BIOPSY SAMPLES (9 POINTS)
實驗三: 法醫學鑑定生物檢體(9分)

Q.3.1. (9 POINTS)Q3.1. (9分)

Based on PCR-RFLP profiling of samples derived from the three patients (P1 – P3) and the three biopsy specimens in Task 2, match X, Y and Z to the patients by filling in the table on the **Answer Sheet**.
比對來自三個病患(P1-P3)的PCR-RFLP圖譜以及在實驗二的生物檢體樣品，推定X, Y, Z和各病患間的對應關係，將之填寫於答案卷的表中。

NOTE! 注意

For solving questions in Tasks 4 and 5, if you did not succeed in genotyping any patient specimens (P1, P2 and P3) in Task 2, you might deduce from profiling their biopsy specimens (X, Y and Z). In those cases, write down X/Y/Z into the column “Patients”. However, there will be a penalty of 1.5 points for each substitution.
回答實驗四和五的問題時，如果你在實驗二中有任丨病人樣品無法成功的完成基因型確定，則在"病人"欄中填入X/Y/Z。每一個這樣的填法將會被扣1.5分

TASK 4: INTERPRETATION OF PATIENTS' GENOTYPES (12 POINTS)
實驗四: 病人基因型的推定(12分)

Q.4.1 (9 POINTS) Q.4.1 (9分)

Indicate the genotypes of the three patients based on the PCR-RFLP profile you obtained from your own digestion with *Kpn*I (NAT2*5 or C481T) and *Bam*HI (NAT2*7 or G857A) by completing the table in the **Answer Sheet**. The genotype of the locus NAT2*6 (G590A) based on the *Taq*I digestion is already given for the three patients.
依據你的限制酶切割所得的PCR-RFLP圖譜，指出每位病人的相關基因型: KpnI用於推定NAT2*5(C481T)；BamHI用於NAT2*7(G857A)，將答案填在表中。表中已提供依據TaqI切割結果所推定的三位病患之NAT2*6 (G590A)基因型。

Q.4.2 (3 POINTS) Q.4.2 (3分)

Indicate the acetylator phenotype of the three patients based on their genotypes you determined in this task (Question 4.1) by ticking (√) in relevant boxes of the table in the **Answer Sheet**.
依據你在Q 4.1所判定這三位病人的基因型，指出他們有關乙醯化的表現型，在答案卷表中的相關格內打勾(√)。

實驗

A Patient P1 病患1
B Patient P2 病患2
C Patient P3 病患3

TASK 5. DETERMINATION OF DRUG DOSAGE RELEVANT TO PATIENTS' GENOTYPES (18 POINTS)
實驗五. 決定對應病人基因型的用藥劑量

Introduction 背景介紹

In 2015, a study performed by Jung AJ and co-workers (*Journal of Drug Design, Development and Therapy; 9: 5433-8*) on 206 patients with TB who received INH at the dose of the standard regimen (5 mg/kg body weight, usually 300 mg INH daily) indicated that 2-hour post-dose serum concentrations of INH were significantly lower in the rapid-acetylators than in the slow-acetylators. A multivariate stepwise linear regression analysis that included the variables of age, sex, body weight, and NAT2 genotype revealed that NAT2 and body weight independently affected INH concentrations ($P < 0.001$), while other variables did not alter INH concentration ($P > 0.05$). According to the regression analysis, the equation that best predicts INH concentration is as follows:
2015年一項針對206個肺結核(TB)病人的研究，這些病人皆接受INH的標準用藥劑量(5 mg/kg體重，通常每天接受300 mg 的INH)，在用藥2小時後，檢驗患者血清中NIH的濃度，發現在快乙醯化能力者的血清INH濃度明顯低於在慢乙醯化能力者血清的INH濃度。丨個包含年齡、性別、體重和NAT2基因型的多變數逐次線性迴歸分析發現: NAT2和體重分別獨立影響INH的濃度($P<0.001$)；而其它變數則對INH濃度沒有影響($P>0.05$)。根據此迴歸分析，對於INH濃度的最佳預測方程式如下:

Serum INH concentration (mg/L) = 13.821 - 0.1 × (body weight, kg) - 2.273 × (number of high activity alleles of NAT2; 0, 1, 2) **(Equation 1)**
血清 INH 濃度 (mg/L) = 13.821 - 0.1 × (體重, kg) - 2.273 × (NAT2的高活性等位基因數目: 0, 1, 2) **(方程式1)**

In this equation, the number of high activity alleles of NAT2 (0, 1 or 2) corresponds to the three phenotypes, slow-, intermediate- and rapid-acetylators, respectively.
在此方程式中，NAT2的高活性等位基因數目(0, 1, 和2)分別對應三種表現型:慢乙醯化能力者、中乙醯化能力者、和快乙醯化能力者

The most effective anti-TB therapy of INH was found when its 2-hour post-dose serum concentration fall within the range of 3.0 – 6.0 mg/L. Based on the concentration of INH shown on the drug label in **Figure 4**, you are to determine the appropriate prescription for patients P1, P2 and P3. Work according to the next two steps (Questions 5.1 and 5.2) and assume all three patients are 70 kg in weight each.
最有效力的對抗TB治療法是讓用藥2小時後血中INH濃度介於3.0-6.0 mg/L。根據圖**4**中INH藥品的標示濃度，你要依循以下二步驟(問題5.1及5.2)去決定病人P1, P2, 和P3的適宜處方，假設三位病人體重都是70 kg。

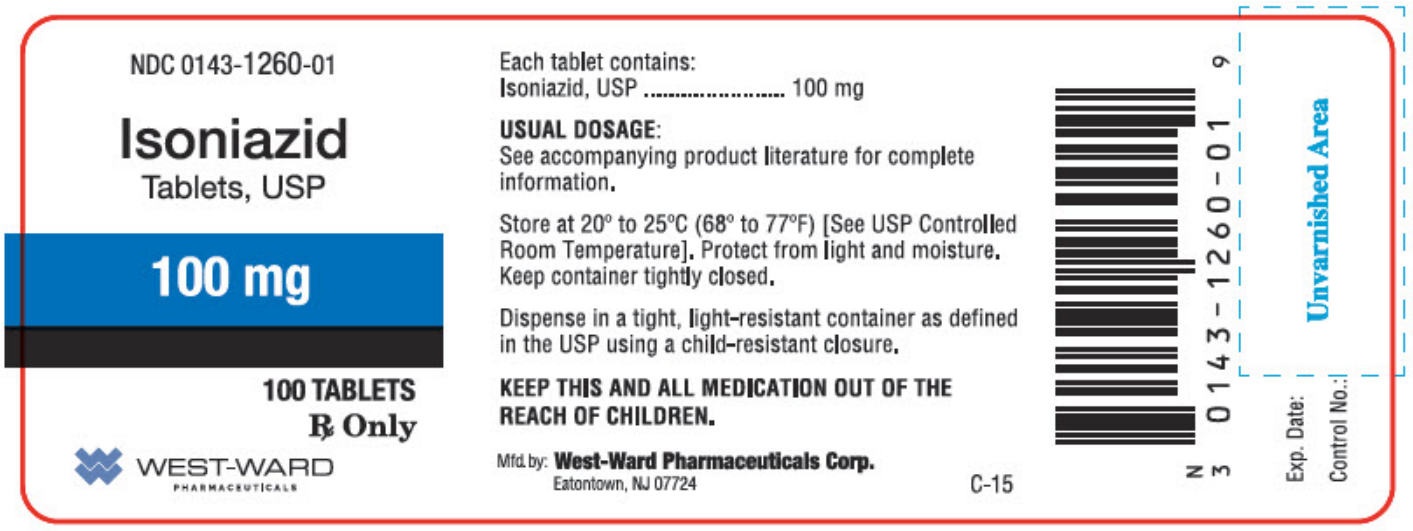


Figure 4. Isoniazid (INH) Drug Label

Figure 4. Isoniazid (INH) Drug Label
INH藥品標示

Q.5.1 (6 POINTS) Q.5.1 (6分)

Presumably each patient takes the daily dose of 300 mg INH, estimate the 2-hour post-dose serum concentration of INH in P1, P2 and P3 based on Equation 1 and their genotypes you identified by completing the table in the **Answer Sheet** (numbers are presented to 3 decimal digits).
假設每位病人每天服用300 mg INH，依據方程式 1和你判定的病患基因型，請估算病患P1, P2, 和P3用藥2小時後的血中INH濃度，將答案填在答案卷之表格中 (取小數點下三位)

Q.5.2 (12 POINTS)

Based on the patients' corresponding *NAT2* genotype determine the least number of drug tablets (shown in **Figure 4)** each patient should be administrated as daily dose to achieve an anti-TB therapy within the most effective range and fill in the table in the **Answer Sheet (**numbers are presented in integer).
依據各病患的NAT2基因型，計算出每位病患每天所需的最有效TB治療劑量。並將每位病人每天應服用的藥片數(整數)寫在圖4表格中。

END OF PRACTICAL EXAM 4!
實作測驗4結束!

Country 國家:
Student Code 學生編號:

27th International Biology Olympiad

July 17-23, 2016
Hanoi, Vietnam

Practical Exam 4　實作測驗四

MOLECULAR BIOLOGY　分子生物學

ANSWER SHEET 答案卷

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

問題**1.1 (12**分**)**

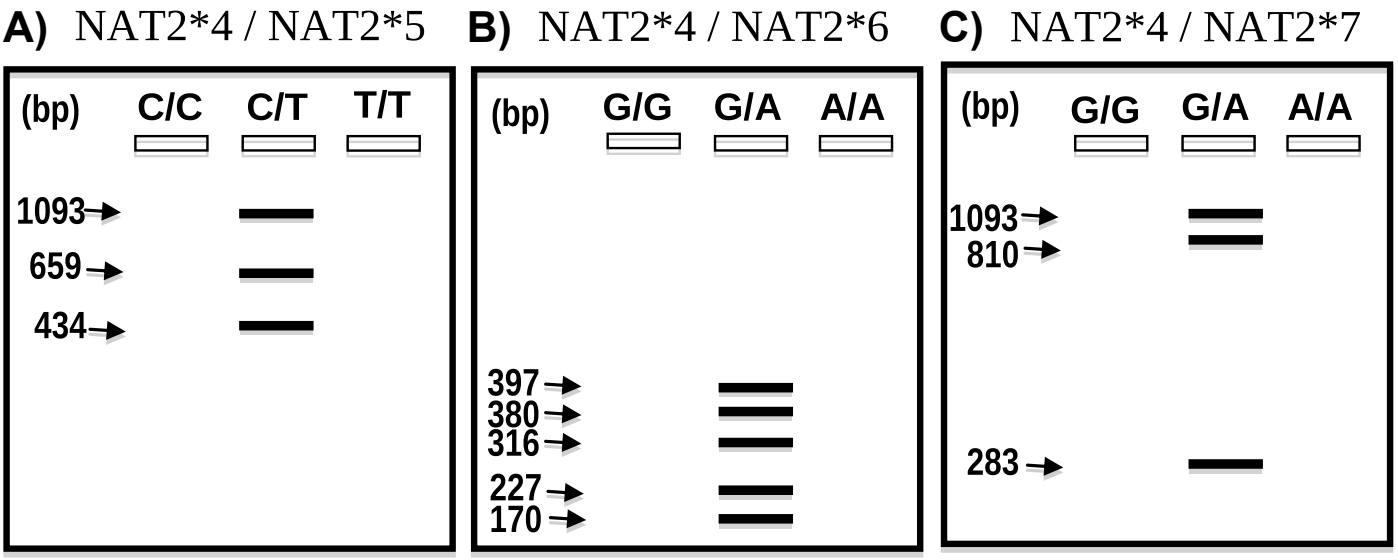


Figure Q.1.1: Expected RFLP pattern of **(A)** homozygous wild-type NAT2*4 and homozygous mutant NAT2*5 (T/T) digested by *Kpn*I, **(B)** homozygous wild-type NAT2*4 (G/G), homozygous mutant NAT2*6 (A/A) digested by *Taq*I, and **(C)** homozygous wild-type NAT2*4 (G/G), homozygous mutant NAT2*7 (A/A) digested by *Bam*HI. **(A,B,C)** Bands in the middle lane represent the respective heterozygous genotypes.

問題1.1圖: 預期之RFLP型態

(A)以*Kpn* I 限制酶切割之同型合子野生型 NAT2*4 及同型合子突變型NAT2*5(T/T)

(B) 以 *Taq* I 限制酶切割之同型合子NAT2*4 (G/G), 同型合子突變型NAT2*6(A/A)

(C) 以 *Bam* HI 限制酶切割之同型合子野生型NAT2*4 (G/G), 同型合子突變型NAT2*7(A/A)

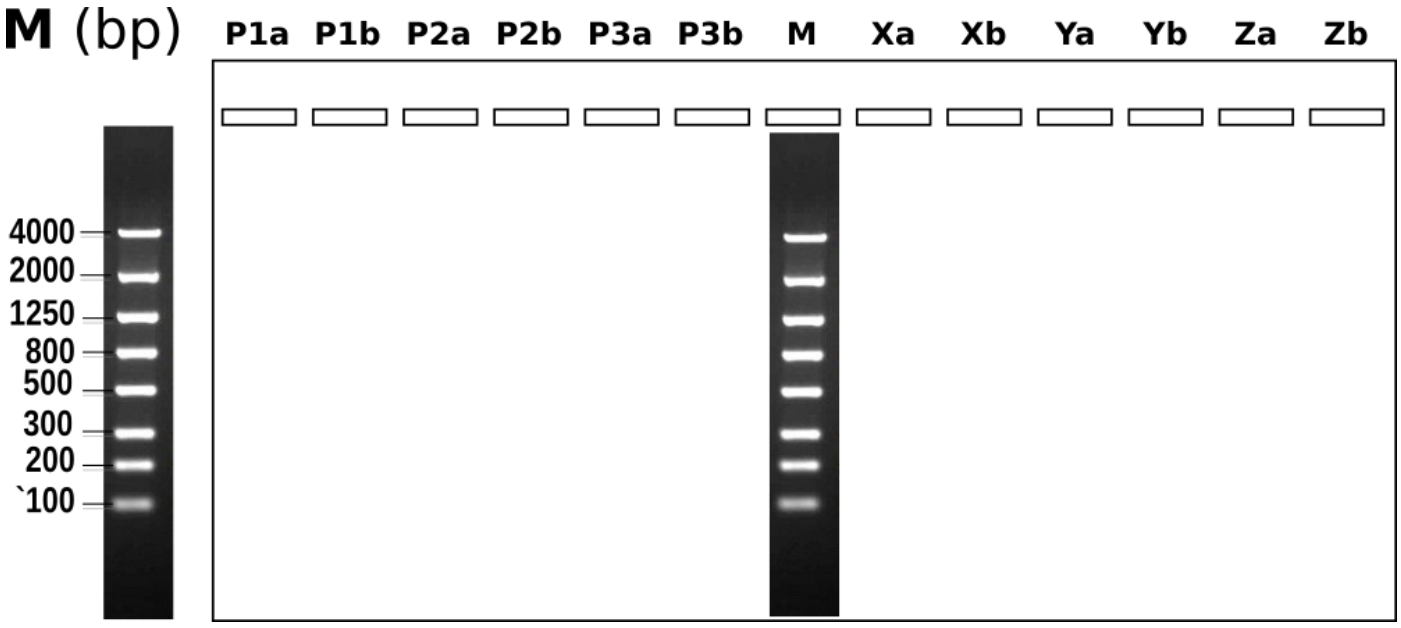
(A,B,C)中間樣品槽的條帶代表其相對應異型合子基因型

Q1.2 (5 POINTS)

問題**1.2 (5**分**)**

Reagents試劑 **(in µL)**	**NAT2*4 / NAT2*5** (labelled **a**) (標示**a**)	**NAT2*4 / NAT2*7** (labelled **b**) (標示**b**)
Sterile water無菌水 (W)		
10 x Restriction Buffer 10x 限制酶緩衝液(BF)		
PCR products of genomic DNA 基因體DNA的PCR產物 (2.0 µg/µL)		
RE 1 *(Kpn*I*)*		
RE 2 *(Bam*HI*)*		
Total volume總體積 (µL)	10	10

Q.2.1. DRAWING OF GEL (18 POINTS)



Q.2.2 PHOTO (26 POINTS)
Q.2.2照片 (26分)

Attach photo of gel here

Q.3.1. (9 POINTS)Q3.1. (9分)

Patients 病人	Biopsy specimens (X, Y or Z) 生物檢體(X, Y, Z)
P1	
P2	
P3	

Q.4.1 (9 POINTS) Q.4.1 (9分)

Patients 病人	Genotype at C481T C481T基因型	Genotype at G590A G590A基因型	Genotype at G857A G857A基因型
P1		G/G	
P2		G/A	
P3		G/G	

Q.4.2 (3 POINTS) Q.4.2 (3分)

	Slow acetylator慢乙醯化者	Intermediate acetylator中乙醯化者	Rapid acetylator快乙醯化者
A			
B			
C			

Q.5.1 (6 POINTS) Q.5.1 (6分)

Patients 病人	**INH concentration (mg/L)** **INH濃度 (mg/L)**
P1	
P2	
P3	

Q.5.2 (12 POINTS)

Patients 病人	**Oral dosage (number of tablets per day)** 口服劑量(每日藥片數)
P1	
P2	
P3	