PRACTICAL 2: BIOCHEMISTRY
實作2:生物化學

Student name:	Student code:	Country:

28th International Biology Olympiad

July 23-30, 2017
University of Warwick
United Kingdom

Practical Exam 2

BIOCHEMISTRY

The exam will start and end with a whistle.

Total points: 78
Duration: 120 minutes

GENERAL INSTRUCTIONS
一般說明

一般說明

In this practical test you have **TWO hours** to do **THREE Questions**.
本場實作考試時間為2小時，需要完成3個問題

You should perform the tasks in the order given here:
你必須依下列順序完成你的任務

Question 1: Analysis of blood markers (11 marks). This section should take approximately 15 minutes to complete. You will be provided with some data to analyse.
問題1:血液分析 (11分)
依據提供的資料進行血液標記分析，需在大約15分鐘內完成

Question 2: Practical determination of kinetic parameters (60 marks). This section should take approximately 90 minutes to complete. You will be generating your own data.
問題2: 動力學參數之實作測定(60分)

你必須操作實驗進行動力學參數之實作測定,，才能有自己的數據，需在大約90分鐘內完成

Question 3: Analysis of genetic markers (7 marks). This section should take approximately 5 minutes to complete. You will be provided with some data.
問題3: 遺傳標記分析(7分)
依據所提供的資料完成遺傳標記分析，需在大約5分鐘內完成，

In this exam you will analyse a patient history through blood marker characteristics, enzyme kinetics and family inheritance of a genetic disorder.
Good luck!
在本實作中，你將透過一位病人的血液標誌特徵、酵素動力學分析，和家族遺傳疾病的遺傳模式，去分析此病人之病史
祝 好運!

Important Information:重要信息：

- Please remember to write your name, your student code and your country in the given boxes.
  請記住在所指定的空格中寫下您的姓名，學生代碼和國家。
- Write your answers in this question booklet. Only the answers given in this question booklet will be evaluated.
  在試卷本內上寫下你的答案。只有在試卷本中的答案會被評分。
- Make sure that you have received all the materials and equipment listed. If any of these items are missing, please raise your Red card immediately.
  先確定你已收到所有表列的材料和設備。如果有任何項目遺漏，請立即舉起您的紅卡。
- During experiments, ensure to handle equipment properly. Any spilled solutions or equipment damaged by you will not be replenished.
  在實驗過程中，確保妥善使用設備。任何洒出的溶液或你損壞的設備將不會被補充 。
- Stop answering and put down your pen immediately when the whistle sounds at the end of the exam.
  在考試結束時的口哨聲響起時，請立即停止回答並放下筆。
- Leave the question booklet on your desk at the end of the exam.
  考試結束時，請將試卷本放在桌上。
- No paper, materials or equipment should be taken out of the laboratory.
  不得將紙張，材料或設備攜出實驗室。
- **An English translation of this paper is available upon request.**
  本試卷本的英文版本可依要求提供。

MATERIALS 實驗材料

Materials
材料

- Ice Box冰盒
- Solution A: 2 x Concentration Reaction Master Mix containing: 50 mM potassium phosphate buffer, pH 7.6; 14 mM $MgSO_4$; 2.64 mM ADP; 40 U Lactate Dehydrogenase; 0.4 mM NADH (10 ml)(in ice box)
  溶液A：2×濃度的反應混合物，含有：50 mM磷酸鉀緩衝液，pH7.6; 14mM $MgSO_4$; 2.64mM ADP; 40 U 乳酸脫氫酶; 0.4 mM NADH（10 ml）(置冰盒中)
- 溶液B：H_2O（10 ml）
- Solution C: 10 mM phosphoenol pyruvate (PEP) in 0.1 M potassium phosphate buffer, pH 7.6 (1.5 ml) in ice box
  溶液C：1.5 ml 的10 mM磷酸烯醇丙酮酸（PEP）配製於 0.1 M 磷酸鉀緩衝液中, pH 7.6 (1.5 ml）(置冰盒中)
- Diluted plasma samples F, M and D (0.5 ml each)
  稀釋的血漿樣品F，M和D（各0.5 ml）(置冰盒中)
- A 20-200 µl pipette 一支20-200µl 微量吸管
- A 100-1000 µl pipette or 200-1000 µl pipette
  一支100-1000µl或 200-1000 µl 微量吸管
- Pipette tips, to suit pipettes 適配微量吸管的微量吸管尖
- 1 ml plastic cuvettes (1 cm path length) x 20
  1 ml 塑料比色管（1 cm 路徑長度）x 20
- Waste tip and cuvette disposal vessel
  放置用過的吸管尖及廢棄比色管的容器
- A visible light spectrophotometer - check the Spectrophotometer Guide sheet, included as a separate document, for instructions on using you spectrophotometer
  一台可見光分光光度計- 檢查分光光度計使用手冊並依指示使用你的分光光度計

  - a cuvette will be placed in sample chamber to indicate the correct orientation.分光光度計樣品槽中已放置有一個比色管用以呈現正確放置方向

- A Cuvette stand 比色管放置架
- Parafilm®square x 30
  30張方形封口膜
- Digital Timer 數位計時器
- 30 cm ruler
  30 公分的尺
- Scientific Calculator
  科學計算器
- Pen 筆
- Pencils 鉛筆

v. 2
Introduction
介紹

Important Background Information重要背景信息

Pyruvate kinase (PK), a 58 kiloDalton (kDa) protein, functions as a homotetramer and has a pivotal role in the glycolytic pathway (Fig. 1), converting its substrate phosphoenol pyruvate (PEP) into pyruvate in a reaction that also generates ATP.
丙酮酸激酶（PK）是一種58千道爾頓（kDa）蛋白，以同源四聚體執行其功能，在糖解作用路徑中扮演關鍵角色（圖1），在反應中將其受質磷酸烯醇丙酮酸（PEP）轉化為丙酮酸，並產生 ATP。

PK deficiency (PKD) is the commonest cause of hereditary non-spherocytic haemolytic anaemia, a group of genetically inherited diseases associated with a net loss of red blood cells. In the PKD disorder, red blood cells are broken down (undergo haemolysis) prematurely, resulting in a shortage of red blood cells (anaemia). In hereditary non-spherocytic haemolytic anaemia, the red blood cells do not assume a spherical shape as they do in some other forms of haemolytic anaemia. Blood analysis can also be helpful in understanding the PKD disease. In addition, the disease is often associated with reticulocytosis; an increase in number of reticulocytes (immature red blood cells).
PK缺乏症（PKD）是造成遺傳性非正常球形細胞溶血性貧血中最常見的病因，是一組與紅血球淨損失相關的遺傳性遺傳疾病。在PKD病症中，紅血球細胞過早分解（血球溶血），導致紅血球（貧血）不足。在遺傳性非正常球形細胞溶血性貧血中，紅血球不像其他一些溶血性貧血症呈現球形。血液分析可有助於了解PKD疾病。此外，該疾病常與網狀紅血球細胞增多症(未成熟紅血球)有關。

PKD is inherited in an autosomal recessive pattern. The parents of an individual with an autosomal recessive condition may, or may not, show signs and symptoms of the condition depending on the alleles that they possess.
PKD是一種體染色體隱性遺傳疾病。此病症個體的父母依其所具有的等位基因情況，可能會或可能不會顯示此病症。

Given equal subunit expression and random association, PK in either simple heterozygotes or compound heterozygotes (heterozygotes where there are two different recessive alleles) is a spectrum of heterotetrameric isozymes (A4, A3B, A2B2, B3A, B4) in a theoretic ratio of 1:4:6:4:1. If B is a variant and A is normal, some 94% of all PK in PKD patients contains one or more mutant subunits; in vivo, non-assembled monomers are prone to protease digestion.
依據次單元基因均等表現及自由組合原則，PK等位基因以簡單異型合子或複合異型合子（異型合子具有兩個不同的隱性等位基因）的型式存在,可形成各種異源四聚體同功酶（A4，A3B，A2B2，B3A，B4）,並以 1:4:6:4:1理論比率存在。如果B是變體，A是正常的，在PKD患者所具有PK中約94％含有一個或多個突變的次單元;在活體細胞中,未組裝的單體易被蛋白酶鎖分解。
It is important to note that specific alleles code for PK proteins that form tetramers, whilst other PK proteinsthat remain as monomers.
須注意的是有些等位基因表現的PK蛋白會形成四聚體，其他等位基因表現的PK蛋白則只以單體存在

Clinical symptoms usually observed in simple heterozygotes, compound heterozygotes and homozygotes are variable, ranging from neo-natal jaundice requiring blood transfusions, to haemolytic anaemias that are self-regulated and show minimal clinical signs. Many of the mutant PKs have been identified on the basis of the biochemical characteristics of the defective enzyme.
通常在簡單異型合子，複合異型合子和同型合子個體中觀察到的臨床症狀是有差異的，從需要輸血的新生兒黃疸到能自我調節的溶血性貧血症並顯示少許的臨床症狀。許多突變型PK基因已可以其表現酵素的生化特徵加以鑑定。

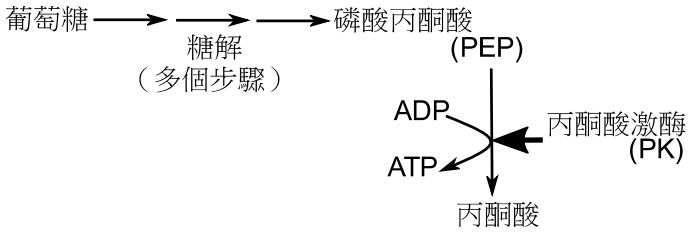


Figure 1: *The role of pyruvate kinase (PK)*
圖1：*丙酮酸激酶（PK）的作用*

A clinical scenario:臨床背景：
Various family members have been diagnosed with non-spherocytic haemolytic anaemia, perhaps associated with PK deficiency. Numerous tests can be conducted including blood work to determine the parameters of the blood composition (haemogram) and an enzyme analysis of plasma specifically for PK activity. Additionally, a genetic analysis including identification of PK proteins from individual members and an analysis of a family pedigree to confirm the likely inheritance pattern can be undertaken.
已有家庭成員被診斷出患有非球形細胞溶血性貧血，可能與PK缺乏有關。可進行許多測試，包括：血液測定以確定血液組成（血液相)的參數；血漿中PK酶活性分析。此外，可以進行遺傳分析，包括：檢測個體成員的PK蛋白組成，以及家族遺傳族譜中分析，以確認其遺傳模式。

You will analyse and collect data from the 3 family members: Father (F), Mother (M) and Daughter (D), in order to determine the family pedigree.
您將分析並收集3個家庭成員的數據：包括父親（F），母親（M）和女兒（D），以確定家庭遺傳圖譜。

QUESTION 1
問題1

Blood Analysis:
Whole blood samples were collected from the Father (F), Mother (M) and their Daughter (D). After blood is taken and analysed the following partial results were obtained (Table 1):
血液分析：
從父親（F），母親（M）及其女兒（D）收集全血樣本。取血並分析後獲得以下部分結果（表1）：

表格1

	病人**F** （父親）	病人**M** （母親）	病人**D** （女兒）
紅血球細胞數（**RBCs**）	5.35×10^{12} /升	3.65×10^{12} /升	3.01×10^{12} /升
白血球數	$7.5x10^{9}$/1	$4.2x10^{9}$/1	$3.4x10^{9}$/1
血紅素濃度	14.4 g / dl	10.5 g / dl	8.5 g / dl
網狀紅血球細胞（**RBCs**%）	1.67%	2.63%	10.46%

Various analyses can be undertaken, including the determination of the haematocrit, the packed red blood cell volume, given as a percentage of total blood volume. The haematocrit is produced by taking up a sample of blood into a micro-capillary tube and centrifuging the sample so that the red blood cells become packed. The percentage of red blood cells is calculated by measuring the packed volume as a function of the total volume in the capillary tube.
可進行下列各種分析，包括測定紅血球細胞容積比，以總血量的百分比呈現。將血液樣品吸入微量毛細管並進行離心，使紅血球積聚於底部，以計算紅血球細胞容積與毛細管中血液總體積百分比。

任務1a

Determine the haematocrit (% packed red blood cells by volume) shown in Figure 2, for each individual. Include these data in the first row of Table 3. This is expressed as a percentage to one decimal place (d.p.).

量測如圖2所呈現的每個個體的紅血球細胞容積比（紅血球細胞體積百分比）。並將這些數據呈現在表3的第一行中。數據以小數點以後第一位數據呈現（dp）。

Figure 2 Haematocrit diagrams from the Father, Mother and Daughter. *The figure shows diagrammatic representations of the haematocrit, depicting a capillary tube of total sample containing red blood cells (left hand side) white blood cells (buffy coat) (between RBC and plasma) and plasma (right hand side).*
圖2 父親,母親及女兒的血球容積比示意圖
本圖顯示毛細管中紅血球體積(左側)及白血球體積(buffy coat,位在紅血球及血漿中間)及血漿體積(右側)

Table 2: *Normal Values for Haemogram of healthy people*

	Male	Female
Haemoglobin (g/dl) 血紅素	13.5 - 17.5	11.5 - 15.5
Haematocrit (%) 紅血球容積比	40.0 – 52.0	36.0 – 48.0
Red blood cell Count (x10^{12}/l) 紅血球細胞數	4.50 - 6.50	3.90 - 5.60
Mean Cell Haemoglobin (MCH) (pg) 平均細胞血紅素量 (pg)	26.0 - 34.0	26.0 - 34.0
Mean Cell Volume (MCV) (fl) 平均細胞容積 (fl)	78.0 – 95.0	78.0 – 95.0
Mean Cell Haemoglobin Concentration (MCHC) (g/dl) 平均細胞血紅素濃度 (g/dl)	30.0 - 35.0	30.0 - 35.0
White Blood Cell count (x10^{9}/l) 白血球細胞數	4.0 – 11.0	4.0 – 11.0
Platelet Count (x10^{9}/l) 血小板數目 (x10^{9}/l)	105 – 450	105 – 450
Reticulocyte Count (%) 網狀紅血球細胞數**(%)**	0.5 – 1.5	0.5 – 1.5
PK activity (U/g Haemoglobin) PK酵素活性 (單位/克 血紅素)	11.8 – 18.6	11.8 – 18.6

Task 1b 任務1b

Using the packed cell volume from Figure 2, and the patient data from Table 1, calculate the following parameters for each patient (F, M and D):
以圖2的PCV值及表1的病人資料計算每位病人的下列參數值

The red blood cell volume, known as the **Mean Cell Volume** (**MCV**),
紅血球平均容積 (MCV)

The mass of haemoglobin per red blood cell, known as the **Mean Cell Haemoglobin** (**MCH**),
每個RBC細胞中所含平均血紅素含量(MCH)

The concentration of haemoglobin per red blood cell, known as the **Mean Cell Haemoglobin Concentration** (**MCHC**).
每個RBC所含的平均血紅素濃度 (MCHC)

The MCV is given in femtolitres (fl) (fl is 1x10^{-15} l) to one d.p. MCH is expressed in picograms (pg) (pg is 1x10^{-12} g) to one d.p.
MCV以femtoliter (fl) (fl= 1 x 10^{-12} g)並以小數點後一位數值呈現

MCHC is the total concentration of Hb in the RBC fraction. This is expressed as g/dl (deci litre) (1x10-1 l) to one d.p
MCHC為總體RBC所含的血紅素總濃度,此數值以 g/dl (dl=1 x 10^{-1} l)表示,並呈現至小數點後一位數值

Write the numbers in Table 3. for the Father (F), Mother (M) and Daughter (D).
請將父親F, 母親M及女兒D之計算數值填入表3中

Show your calculations for each parameter, in Box 1 for Father (F)),
將(父親F)的每一參數之計算分別填入Box 1中

Box 1: Calculations for patient F (Father) ***(4 marks)***
病人F(父親)的計算
(總分4分)

Element	Show your working here
Haematocrit	
MCV	
MCH	
MCHC	

Table 3 ***(4 marks)***
表3 (4分)

	Sample F (Father)	Sample M (Mother)	Sample D (Daughter)
Haematocrit (%)			
MCV (fl)			
MCH (pg)			
MCHC (g/dl)			

Further analysis 進一步分析
Anaemias are classified, according to the size of the red blood cell, as being either normocytic (normal MCV), macrocytic (increased MCV) or microcytic (decreased MCV). Microcytic anaemias are also often described as being hypochromic based on peripheral whole blood smear examination. The optical properties of the small, thin microcytes make them appear hypochromic on the blood smear, while the haemoglobin concentration remains in the normal range (microcytic; hypochromic anaemia).

貧血症依據RBC細胞大小分成,正常細胞, 巨細胞及小細胞型貧血症, 小細胞型貧血症又依據其周邊血液抹片光學鏡檢呈現小而薄的小RBC細胞,及低血色素含量的光學特性,但其血紅素濃度仍在正常範圍 (小細胞型: 低血色素型貧血)

Normochromic blood smears show no change in optical properties on the blood smear with the haemoglobin concentration remaining in the normal range.
正常血色素血液抹片顯示其血色素光學特性未變,且血紅素濃度在正常範圍

Table 2 gives the normal ranges for MCV, MCH and MCHC.
請估算MCV, MCH及MCHC的正常數值範圍

Task 1d 任務 **1d**
Using the values you have obtained (included in Table 3.), establish the classification in terms of size of blood cells and levels of haemoglobin of the blood of sample F, M and D.
請依據表3中所呈現的數值估算樣品F,M及D的RBC大小及血紅素含量,並加以分類

You should answer from one of the following options for each patient (N.B. you can use any of the options more than once):請以下列所示三種型式回答對每位病人的描述(每種型式都可被重複使用)

A. Macrocytic, normochromic
B. Microcytic, hypochromic
C. Normocytic, normochromic

(3 marks)
(3分)

Patient	Classification (A, B or C)
Father (F)	
Mother (M)	
Daughter (D)	

QUESTION 2
問題2

Assay of pyruvate kinase activity
Biochemistry can be utilised to determine properties of well characterised enzymes by studying the variation in their kinetics. Enzyme activity is usually determined by monitoring the disappearance of a substrate or the appearance of a product, often using changes in spectrophotometric properties of the reaction mixture.

丙酮酸激酶活性分析
透過研究酵素活性的動力學變化，可以決定一酵素的特性。酵素的活性一般是以量測受質的減少和產物的增加來推算，常常可以藉由反應混合物的分光光度數值變化而得知

PK is conveniently assayed by a **coupled assay** in which the product of the reaction, pyruvate (Equation 1), is used as substrate for the NADH-linked enzyme lactate dehydrogenase (LD) (Equation 2), which is added to the reaction mixture in sufficient amounts to convert all of the pyruvate produced during the reaction to lactate. The reaction can be monitored by following the decrease in absorbance at 340 nm over time. The equations for the coupled reaction is shown (Fig. 3).

PK活性可以使用雙合反應分析(如圖3)：反應1的產物，丙酮酸(pyruvate)可以成為反應2的受質，加入足量的與NADH結合之乳酸脫氫酶(LD)，可以將所有的丙酮酸轉化為乳酸，此反應可以透過量測340 nm的減少來偵測。

(equation 1) Phosphoenol pyruvate (PEP) + ADP $\xrightarrow{PK}$ ATP + pyruvate

(equation 2) Pyruvate (from equation 1) + NADH $\xrightarrow{LD}$ NAD^+ + lactate

Figure 3. *Pyruvate kinase and Lactate dehydrogenase-catalysed breakdown of Phosphenol pyruvate (PEP) (Equation 1) and Pyruvate (Equation 2)*

An enzyme's kinetic properties can be determined by varying the PEP substrate concentration (Equation 1).
酵素的動力學特性可以藉變動反應的PEP受質的濃度來決定

Aims of the experiment
To determine the functionality of PK from the three patient plasma samples, Father (F), Mother (M) and the Daughter (D).
實驗目標
分析來自父(F)、母(M)和女兒(D)血漿樣品中PK的功能性

- Determination of activity of PK in patient plasma samples through the use of the coupled assay.
  以雙合反應分析病人血漿內PK的活性
- Calculation of the kinetic properties of PK in absolute units.
  計算PK的動力學特性(絕對單位)

Methods: pyruvate kinase assay
Before starting the assay, please note the following:
方法：丙酮酸激酶分析
分析前，注意以下事項：

1. Solution A, C and patient samples F (father), M (mother) and D (daughter) should be kept on ice, whilst Solution B (H_2O) should be kept at room temperature.
   溶液A、溶液C以及父(F)、母(M)和女兒(D)的樣品必須放在冰上；但溶液B (H_2O)必須放在室溫
2. The spectrophotometer should be blanked against water (provided) at 340 nm.
   在使用波長340 nm 分光光度計時，要先以水(有提供)做空白設定
3. Make up the reaction mix by pipetting the reagents shown in Table 4 directly into a plastic 1 ml cuvette. You should add all the components *except* the plasma sample, *then* add the plasma sample last when you are ready to start the reaction.
   先將表4中除了血漿樣品之外的所有反應成分配成反應混合液，吸排混和後，分別加至1 ml光度計小管。當要開始反應時，再加入血漿樣品。

Table 4. Pyruvate kinase assay components
表4 丙酮酸激酶分析所使用之成份配方

You are provided with the following reagents 提供之藥品	
Solution A 溶液**A**	2x stock concentration Reaction Master Mix (10 ml) (the working concentration for the Reaction Master Mix is 1x) 2倍濃度的主要反應混合液 (10 ml) (反應需在1倍濃度下進行)
Solution B 溶液**B**	H_2O (10 ml)
Solution C 溶液**C**	10 mM phosphoenol pyruvate (PEP) (1.5 ml) 10 mM 磷酸烯醇丙酮酸(PEP) (1.5 ml)
Diluted patient plasma labelled F (Father), M (mother), or D (Daughter) 稀釋的病人血漿樣品，標示父(F)、母(M)、或女兒(D)	Diluted plasma from each patient (0.5 ml each) 稀釋的樣品(每種0.5 ml)

Task 2a
Calculate the reaction volumes for FIVE concentrations of PEP (Solution C) (chose FIVE concentration ranging from 0.2 mM to 1.5 mM). Show an example of your calculations for the highest concentration in Box 2.

任務2a
自從0.2 mM 到1.5 mM的範圍內，自己選擇五種PEP (溶液C) 的反應濃度，並計算對應五種PEP反應濃度的時的使用積體量。將其中最高濃度的計算過程寫在Box 2

PEP will drive the reaction and allow the determination the Pyruvate Kinase activity in each plasma sample (from Father (F), Mother (M) and Daughter (D)).
PEP會啟始反應，藉以測定各血漿樣品中丙酮酸激酶的活性

Box 2 ***(1 mark)***
Box 2 (1分)

Show an example of your workings for the calculation of volumes to be used of the highest PEP concentration. 舉例寫出當使用最高**PEP**濃度反應時，如何計算出使用體積

Task 2b
Write the volumes used to make the reaction mixes into the blank spaces in Table 5; note that these will be the same for each plasma sample (F, M and D).
任務2b
在表5空格內，填入配製反應混合液中各成份的體積；注意：每一血漿樣品(F, M, D)的反應配方應該相同

Table 5, Reaction Volumes. You should write the substrate concentrations, PEP [S], you have decided to use in the first row at the top of each column, and the volumes of each solution in the respective boxes. ***(3 marks)***
*表5 反應體積，先決定第一列的各**PEP**濃度，再決定各對應成份的體積 (3分)*

Concentration 濃度	**1**	**2**	**3**	**4**	**5**
PEP concentration					
Solution A (µl)					
Solution B (µl)					
Solution C (µl)					
Plasma 血漿	50 µl	50 µl	50 µl	50 µl	50 µl
Final volume 總體積	**1000 µl**	**1000 µl**	**1000 µl**	**1000 µl**	**1000 µl**

The PK activity Assay
Mix contents by inverting of the cuvette (making sure there is a piece of parafilm held in place over the opening using your thumb or forefinger), take the A_{340} reading at time zero, then record the A_{340} at 30 second intervals for 90 seconds. Record your absorbance values to the number of decimal places as displayed on your spectrophotometer.

PK活性分析
將比色小管用封口膜封好，大拇指按住開口端，反轉混合反應液，測A340，記錄為0時(time 0)數值，而後每30秒測記一次，共90秒。
For each sample you need to determine the initial rate of reaction $\Delta A/\Delta t$ (time expressed in minutes) for the various concentrations of PEP ([S]) you used. Using your time zero value and another value of your choice. Highlight the time points which you have used to calculate the rate by circling the values.

你必須定出每一種樣品的$\Delta A/\Delta t$ (時間以分鐘表示)，計算對每一種PEP濃度 ([S])的開始變化率($\Delta A/\Delta t$)。以時間點為零時的數值及另一時間點的數值進行計算,並將你計算所使用範圍的時間點用筆圈出

Task 2c
Perform the experiment as described and record the absorbance readings using the tables provided (Tables 6.1, 6.2 and 6.3).

任務2c
如上述步驟執行實驗，並將吸光值記錄在表6.1, 6.2和6.3

Table 6.1 Sample F (6 marks)
表6.1 樣品F (6分)

Concentration 濃度	**1**	**2**	**3**	**4**	**5**
[S]					
Absorbance at 0s 反應**0**秒時的吸光值					
Absorbance at 30s 反應**30**秒時的吸光值					
Absorbance at 60s 反應**60**秒時的吸光值					
Absorbance at 90s 反應**90**秒時的吸光值					
Rate (ΔA/min) 變化率**(ΔA/min)**					

Table 6.2 Sample M (6 marks)
表6.2 樣品M (6分)

Concentration 濃度	**1**	**2**	**3**	**4**	**5**
[S]					
Absorbance at 0 s 反應**0**秒時的吸光值					
Absorbance at 30 s 反應**30**秒時的吸光值					
Absorbance at 60 s 反應**60**秒時的吸光值					
Absorbance at 90 s 反應**90**秒時的吸光值					
Rate (ΔA/Δt) 變化率**(ΔA/Δt)**					

Table 6.3 Sample D (6 marks)
表6.3樣品D (6分)

Concentration 濃度	**1**	**2**	**3**	**4**	**5**
[S]					
Absorbance at 0 s 反應**0**秒時的吸光值					
Absorbance at 30 s 反應**30**秒時的吸光值					
Absorbance at 60 s 反應**60**秒時的吸光值					
Absorbance at 90 s 反應**90**秒時的吸光值					
Rate (ΔA/Δt)(min^{-1}) 變化率					

Calculation of Kinetic Parameters
It is possible to express a change in absorbance over time as a change in product/substrate concentration. For each initial rate, ΔA/Δt, calculate the initial velocity (v_0) (ΔConc/Δt) of the reactions for each PEP substrate concentration ([S]). The absolute units for velocity should be given as μmol min^{-1} using the Beer-Lambert law. The Beer-Lambert law (or Beer's law) is the linear relationship between absorbance and concentration of an absorbing species.
計算動力學參數
我們可以將吸光值的變化以產物/受質濃度的變化。對每一PEP受質濃度([S])，從 起始變化率ΔA/Δt，計算反應初速 (v_0) (ΔConc/Δt)，依Beer-Lambert law，以μmol min^{-1} 為單位。Beer-Lambert law是吸光值和濃度間的線性關係

The equation is: A = εlc
其關係式為: A = εlc

A = Absorbance
A = 吸光值

ε = molar extinction coefficient
ε = 莫耳吸收係數

l = path length of light
I = 吸光路徑厚度

c = concentration
c = 濃度

The reaction in this experiment measures the conversion of NADH, which has a molar extinction coefficient of 6220 L mol^{-1} cm^{-1}, to NAD^+ (which has a negligible absorbance at 340 nm), and therefore the conversion of PEP to lactate. As there is a 1:1 molar ratio of components the conversion of NADH is therefore equal to the conversion of PEP. The cuvette has a path length of 1 cm.
本實驗的反應是量測NADH轉化為NAD+的變化，其莫耳吸收係數是6220 L mol^{-1} cm^{-1}。NAD+在340 nm波長之吸光值可被忽略不用考量。反應的另一轉化是PEP變成乳酸，因為二種轉化是1:1莫耳比，所以NADH的轉化等同於PEP的轉化。光度計小管的吸光途徑為 1 cm。

Task 2d
Calculate the initial velocity (v_0) and write your answers in the tables below (Table 7.1, 7.2 and 7.3) for each patient (Father (F), Mother (M) and Daughter (D)). Your values should be given to the nearest 3 decimal places (d.p.).
對父(F)，母(M)和女兒(D)分別計算反應初速 (v_0)，將答案寫在以下的表7.1, 7.2和7.3，將你的答案以最接近的小數點後3位數值呈現

Table 7.1 Patient F (1 mark)
表7.1 父 F (1分)

[S]	v_0

Table 7.2 Patient M (1 mark)
表7.2 母M (1分)

[S]	v_0

Table 7.3 Patient D (1 mark)
表 7.3 女兒 D (1分)

[S]	v_0

Calculation of enzyme parameters K_M and V_{max}
酵素K_M及V_{max}參數之計算

By using the different rates of reaction for differing substrate concentrations two enzymatic parameters can be determined: K_M and V_{max}. These two parameters can be estimated by the data in one of two ways: a Michaelis-Menten plot (Figure 4 A) or a Hanes-Woolf plot (Figure 4 B).
兩酵素之K_M and V_{max} 參數可以利用不同受質濃度進行不同反應速率之測定來加以量測,此兩種參數可以下述兩種數據做圖方式來估算:繪製 Michaelis-Menten plot (如圖4A) 或Hanes-Woolf plot(如圖4B)

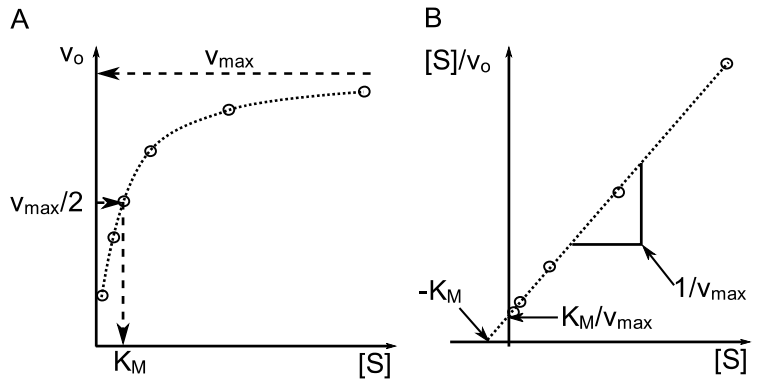
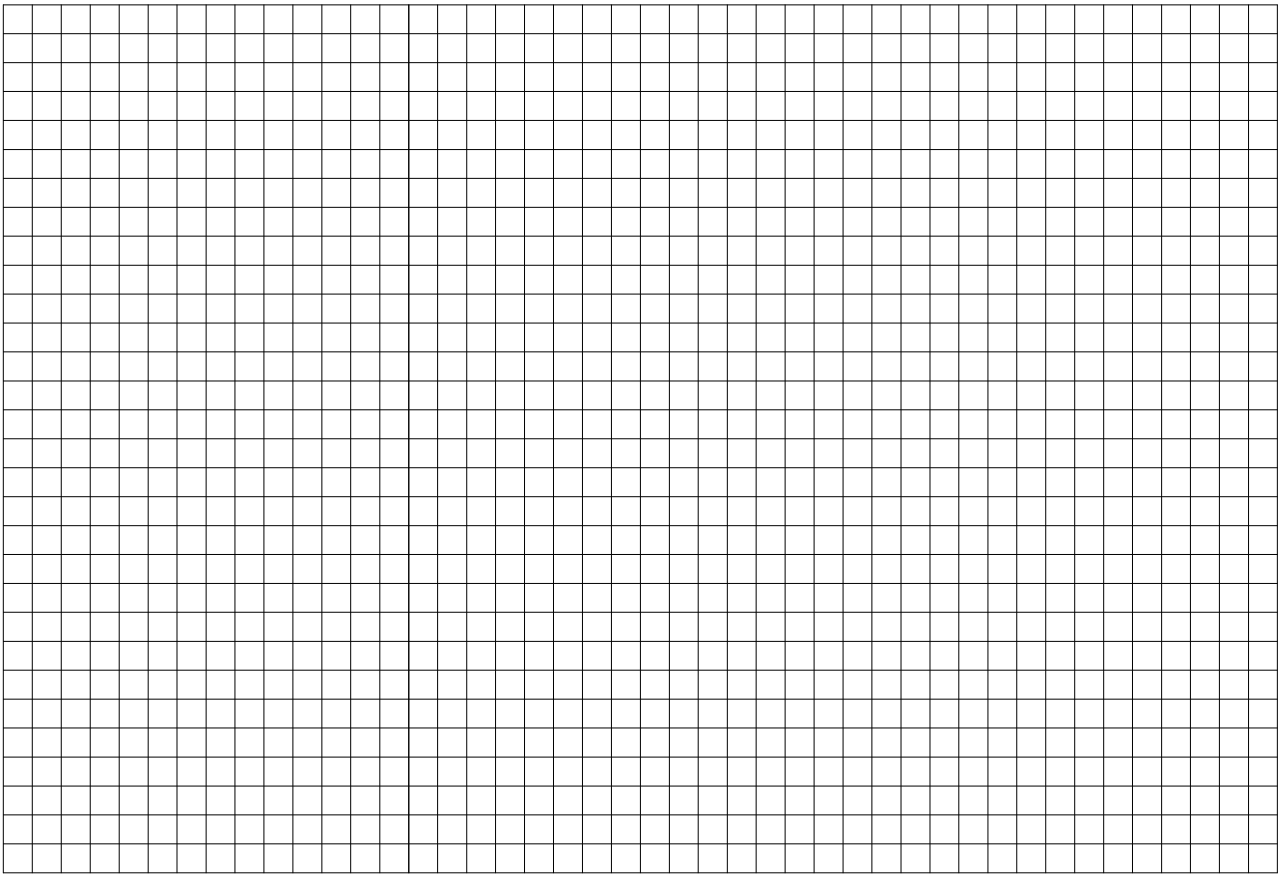


Figure 4. Plotting kinetic data. *動力學數據作圖*
A. *Michaelis-Menten plot of initial reaction rate (v_0) over substrate concentration [S]. K_M is the substrate concentration that gives half maximal reaction rate. V_{max} is the maximal rate where [S] is saturating.*
A.以初反應速率(V_0)及受質濃度[S]繪製 Michaelis-Menten plot.
K_M為達1/2最大反應速率時的受質濃度, V_{max}為當受質飽和時的最大反應速率
B.*Hanes–Wolf plot of the ratio of the substrate concentration and initial rate ([S]/v_0) over the substrate concentration. K_M can be determined from the x-axis intercept of the best linear fit to the data points, and V_{max} can be determined from the slope.*
B. 以 受質濃度與初始反應速率比值([S]/V_0)及受質濃度[S]繪製Hanes–Wolf plot, K_M 為與數據參數點最適直線交叉之x-軸數值來決定, V_{max}由斜率決定

Task 2e
任務2e

Use your data to construct a Michaelis-Menten plot of v_0 over [S] for each patient (F, M and D) on the graph area below. You should draw one graph with three data plots; one each for Father (F), Mother (M) and Daughter (D). Use a cross (X) to mark data from the Father (F), a circle (O) to mark data from the Mother (M) and a triangle (Δ) to mark data from the Daughter (D).

依據所得數據,以每位病人(F, M及D) 的 V_0 / [S] 在下列方格紙中個別建構一個 Michaelis-Menten plot 圖,可用一個圖呈現3個病人父親F (以X標誌表示), 母親 M (以O標誌表示)及 女兒 D(以Δ標誌表示)之數據資料,



Michaelis-Menten plot
(10 marks)
(10分)

Task 2f 任務**2f**
Using the three data plots, estimate Vmax and K_M; write the estimates in the table below using the correct units and to the nearest 2 decimal places (d.p.), for each sample from the graph you have drawn.

請依據三組數據圖，估算V_{max}和K_M,將所估算的數值填入下面表格中,請使用正確的單位,並以小數點後2位數值填入下表中。

(6 marks)
(6 分)

Patient	V_{max}	K_M
Father (F)		
Mother (M)		
Daughter (D)		

Task 2g (3 marks: one mark for each completed table)
任務 **2g (3分: 每完成一表格得1分)**
Determine [S]/v_0 for each substrate concentration and each sample and write your answers in the tables below for each patient (Father (F), Mother (M) and Daughter (D)).Your values should be given to to the nearest 1 decimal places (d.p.).

測定每一受質濃度的 [S]/v_0 並將每位病人(父親 F, 母親 M及 女兒 D)的答案填入下列表格中,答案數值請呈現至小數點後1位

Patient F (1 mark)
病人F (1分)

[S]	$[S]/v_0$

Patient M (1 mark)
病人M (1分)

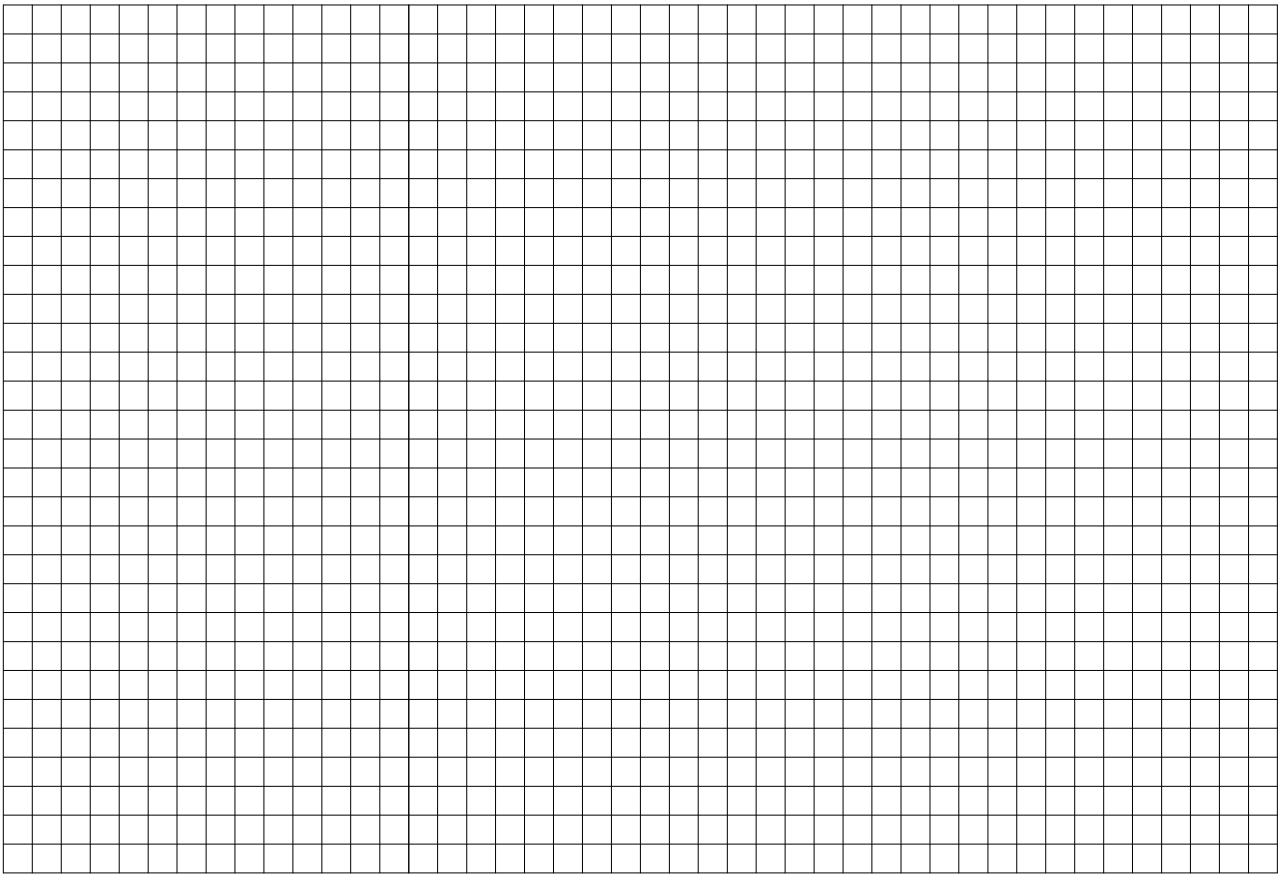
[S]	$[S]/v0$

Patient D (1 mark)
病人D(1分)

[S]	$[S]/v0$

Task 2h 任務 2h

Plot [S]/v_0 over [S] for each patient (F, M and D) on the graph area below. You should draw one graph with three data plots; one each for Father (F), Mother (M) and Daughter (D). Add a line of best fit for each data plot. Use a cross (X) to mark data from the Father (F), a circle (O) to mark data from the Mother (M) and a triangle (Δ) to mark data from the Daughter (D).
請以每位病人(F, M及D)的 [S]/V_0 及 [S] 數據作圖呈現在下圖中,以3 數據(F, M 及 D)做成一圖形,並繪製一條最適合3組數據資料的線
病人父親F (以X標誌表示), 母親 M (以O標誌表示)及 女兒 D(以Δ標誌表示)



Hanes-Woolf plot
(10 marks)
(10分)

Task 2i 任務2i

Calculate V_{max} and K_M for each patient and include in the table below using the correct units to two decimal places (d.p.)

計算每個患者的V_{max}和K_M ，並填在下表中, 並以正確的單位呈現數據至小數點以下2位數

(6 marks)
(6分)

Patient	V_{max}	K_M
Father (F)		
Mother (M)		
Daughter (D)		

QUESTION 3
問題3

Genetic Analysis and Diagnosis
遺傳分析與診斷

Blood samples from the father, mother and daughter were sent to a clinical testing laboratory for further analysis. Proteins were extracted from whole blood, before separation on a polyacrylamide denaturing electrophoresis gel. The samples were visualised, after transferring the proteins to a nitrocellulose membrane (western blot), by detection using enzyme conjugated anti-PK antibodies and subsequent chemiluminescence. The resulting visualisation of the PK protein content is shown in the image in Figure 5. ;Please refer to the Important Background Information to help interpret the figure. ;

將父、母和女兒的血液樣品送至臨床檢測實驗室分析：萃取全血的蛋白質，以變性電泳分離、轉漬至硝化纖維膜，使用已結合酵素之抗PK抗體去偵測PK蛋白，並以化學冷光法觀測，結果如圖5; 並請參考重要背景資料以有助於圖中資料數據之解讀及演譯

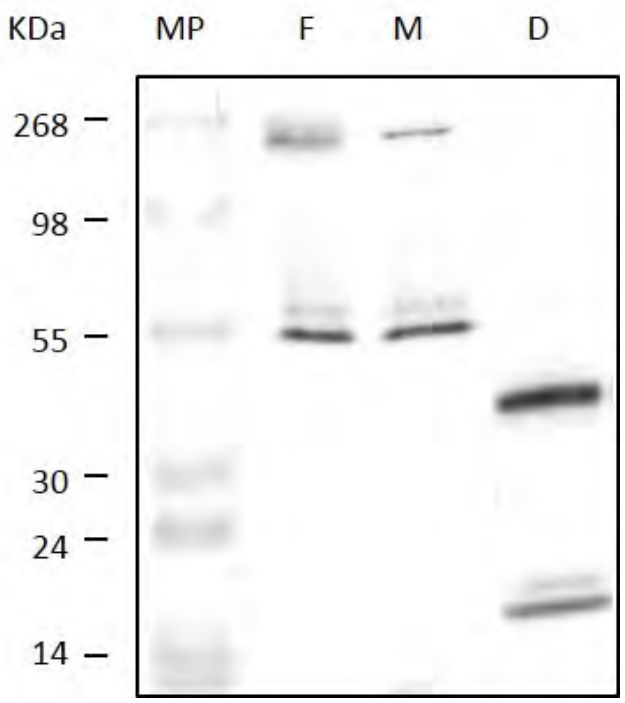


Figure 5. Immunoblot of PK variants in the affected family. *Red blood cell lysates from the father (F), the mother (M) and the daughter (D) were separated by Polyacrylamide gel electrophoresis (PAGE), imtransfered to membrane (western blot), and probed with anti-PK antibodies (immunoblotted. Sizes and migration of marker proteins (MP) are indicated on the left.*
圖5.免疫轉染此受測家庭的PK變異蛋白質。父、母和女兒的紅血球萃取物以PAGE電泳分離，並以抗PK抗體進行免疫(西方墨點)偵測。標記蛋白(MP)左側的數字分別標示其大小。

Task 3a
Complete the table below, indicating which size protein bands are visible for each patient (F, M or D), as either ‘present’ or ‘not present’. Use the letter P to mark present, and NP to mark not present.
任務3a
在下表內紀錄圖5中每一病人(父F、母M和女兒D)之可被偵側到的蛋白質：對應表中各蛋白分子量，寫P代表存在，寫NP代表不存在

Task 3b
Indicate on the table whether any of the three patients (F, M or D) contain a PK tetramer by marking as either ‘present’ or ‘not present’. Use the letter P to mark present, and NP to mark not present.
在下表內指出每一病人(父F、母M和女兒D)是否具有PK四聚體：寫P代表存在，寫NP代表不存在

(5 marks)
(5分)

Molecular weight 分子量	Patient F 父	Patient M 母	Patient D 女兒
232 kDa			
58 kDa			
40 kDa			
18 kDa			
PK tetramer **PK四聚體**			

The clinical testing laboratory also received samples from siblings and other family members and analysed the PK activity of these individuals. In the family, the daughter has two further brothers. (Table 8).
此臨床檢測實驗室也檢測了病人家中其它成員的PK活性。女兒病人有二個弟弟 (表8)

The PK K_M data for other family members is also shown in Table 8.
家中其他成員的PK K_M資料也列在表8

Task 3c
Write your values for the K_M, derived from the Hanes-Woolf calculation, for the Father, Mother and Daughter in the table for your convenience (**zero marks**).

任務3c
為方便你答題，先在表中寫下以 Hanes-Woolf 算式得到的父、母和女兒的K_M值(不計分)

Table 6. *K_M [PEP] for subjects in the extended family; the Proband is a person serving as the starting point for the genetic study of a family.*
表6 家中成員的K_M [PEP]值，原發病患(Proband)是本病例家庭遺傳研究的稱謂基準點

Subject 家庭成員	K_M [PEP]
Daughter (IV-3) (Proband) **女兒(IV-3)(原發病患)**	
Aunt (III-6) **小阿姨(III-6)**	0.45
Grandfather (II-2) **外公(II-2)**	0.40
Great Grandmother (I-1) **外公的媽媽(I-1)**	0.40
Mother (III-3) **媽媽(III-3)**	
Brother (IV-4) **大弟(IV-4)**	0.48
Brother (IV-5) **小弟(IV-5)**	0.35
Father (III-4) **爸爸(III-4)**	
Grandmother (II-1) **外婆(II-1)**	0.20
Aunt (III-2) **大阿姨(III-2)**	0.15
Uncle (III-5) **舅舅(III-5)**	Unknown/Unavailable 無資料

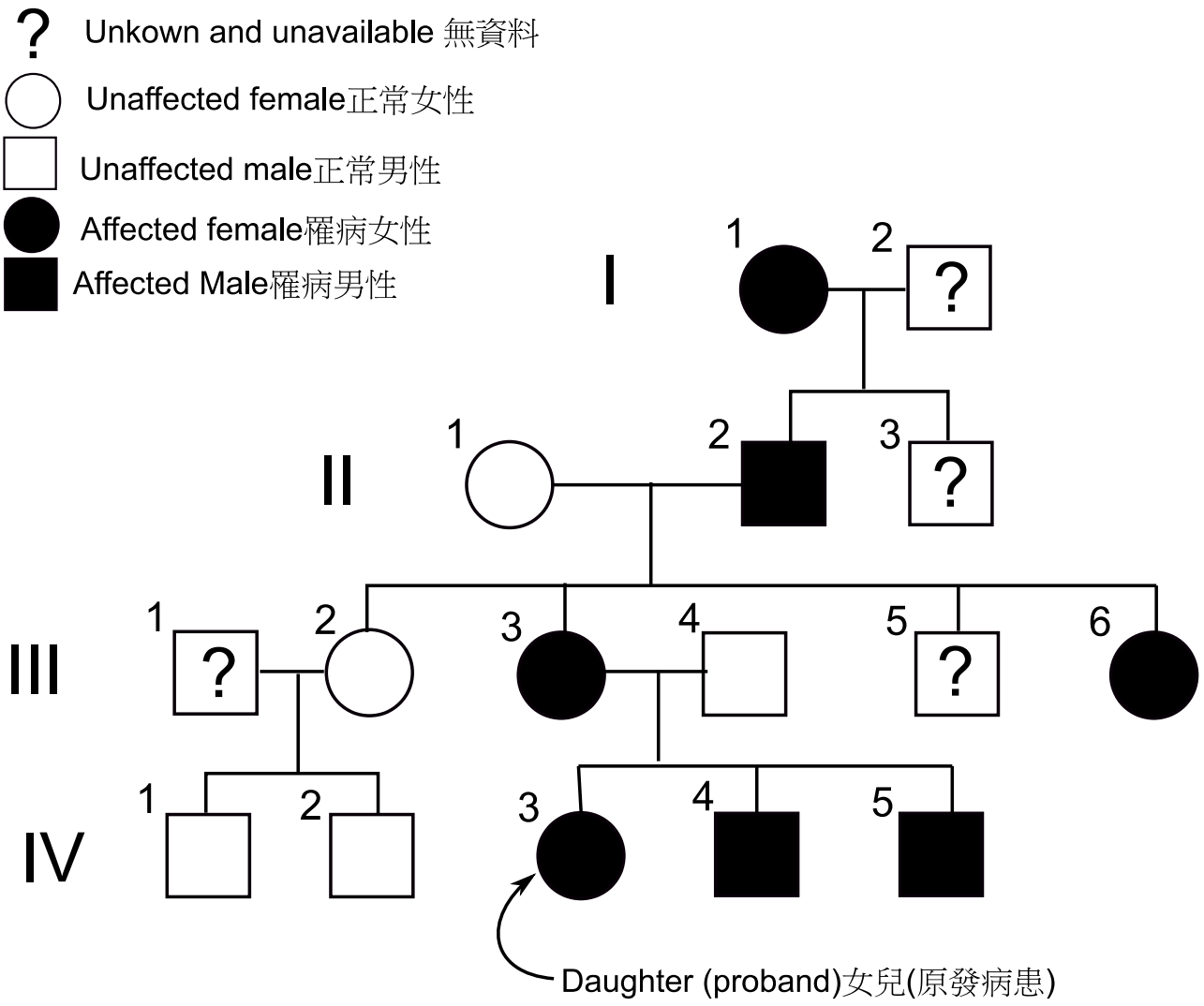


Figure 6. Family Pedigree. *圖6 族譜*

Task 3d (1 mark for the correct answer; you cannot gain a mark for Task 3e unless you have answered Task 3d correctly).

Indicate, by drawing a circle around your choice of either A, B or C, father III-4's genetic PK gene arrangement in terms of:
任務3d (答對得1分；如果沒答對，任務3e不能計分)

爸爸(III-4)的PK基因組成為何? 圈選你的答案 (A、B或C)

A. Simple Heterozygote- (Aa_1, Aa_2, Aa_3)
簡單異型合子(Aa_1, Aa_2, Aa_3)

B. Compound Heterozygote- (a_1a_2, a_1a_3, a_2a_3)
複合異型合子 (a_1a_2, a_1a_3, a_2a_3)

C. Homozygous (AA)
同型合子(AA)

Task 3e (1 mark for the correct answer; you can only receive a mark if you have answered Task 3d correctly).
Indicate, by drawing a circle around your choice of either A or B, brother IV-5's genetic PK gene arrangement in terms of:

任務3e(答對得1分，要答對任務3d，本題才計分)
小弟IV--5的PK基因組成為何? 圈選你的答案 (A或B)

A. Simple Heterozygote
簡單異型合子

B. Compound Heterozygote
複合異型合子

END OF EXAM
試卷結束