Student Code:

24th International Biology Olympiad

14th-21st July, 2013

Bern, Switzerland

BERN 2013 International Biology Olympiad

Practical Exam 1

實作 1

Molecular Cell Biology

分子細胞生物學

Total points: **100**

總分：100

Duration: **90 minutes**

時間：90 分鐘

Dear participants,

This test consists of two tasks:

本實作題包括三部分：

Please write your student code into the box on the title page.

請在封面空格處寫下你的學生編號

There is no separate answer sheet. Please fill in your answers into the specific answers boxes indicated with a gray background. **Only answers given inside these boxes will be evaluated.**

本實作題沒有另附答案卷，請將答案寫在試題內指定的灰色答案格中，只有寫在答案格中的答案才計分

The answers have to be given either with a tick (√) or with Arabic numbers. The numbers "1" and "7" can look very similar in handwriting. To make sure that those two numbers can be well distinguished by the IBO staff, please write them as you normally would into the following box.

答案設計以勾選方式**(√)**或是數字呈現，因為數字 **"1"**和 **"7"**可能會被誤認，為使 IBO 試務人員能正確辨識你的答案，請在下方指定方格內寫下你平常的**"1"**和 **"7"**手寫體

1 =		**7 =**	

Stop answering and **put down your pen IMMEDIATELY** when the bell rings at the end of the exam. Put the entire protocol with all the answers back into the exam envelope.

考試結束鈴響時，立即放下筆停止作答，將試卷放入信封內

Material and equipment

材料和器材

Make sure that you have received all the materials and equipment listed for each task. If any of these items are missing, please raise your hand.

確定你有下列全部的材料和器材，如果缺少任何項目，立即舉手

Equipment

器材

- Water bath at 37°C (used in common)
  37°C 水浴槽(共用)
- 1 Micropipette P1000
  1 支 P1000 微量吸管
- 1 Micropipette P200
  1 支 P200 微量吸管
- 1 Micropipette P20
  1 支 P20 微量吸管
- 1 Box of pipette tips for P1000
  1 盒微量吸管的滴管尖(P1000 使用)
- 1 Box of pipette tips for P200 and P20
  1 盒微量吸管的滴管尖(P200 和 P20 使用)
- 1 Eppendorf holder
  1 個微量離心管架
- 1 Tube holder
  1 個試管架
- 1 Solid waste container
  1 個固體廢棄物容器
- 1 Liquid waste tube **[LW]**
  1 個液體廢棄物容器 **[LW]**
- 1 Polystyrene (styrofoam) box filled with ice
  1 個裝滿冰的冰桶
- 1 Timer
  1 個計時器
- 1 Marker
  1 隻馬克筆

- 1 Microscope
  1 個顯微鏡
- 3 Cell counting chambers
  3 個細胞計數槽玻片組
- 3 Microscope slides
  3 個載玻片
- 3 Cover slips
  3 個蓋玻片
- 21 Eppendorf tubes
  21 個微量離心管
- 1 Magnetic Eppendorf holder
  1 個微量離心管磁力座
- Blank paper
  空白紙
- 1 Flag to call an assistant
  1 個尋求協助旗
- 1 Yellow sheet labeled with your student code
  1 張標記有學生編號的黃色紙

Chemicals

化學藥品

- 1 Eppendorf tube with magnetic beads **[MB]**
  1 個裝有磁珠的微量離心管**[MB]**
- 1 Tube with phosphate buffer **[PBSB]**
  1 個裝有磷酸基緩衝液的離心管**[PBSB]**
- 1 Eppendorf tube with Fixation Buffer **[FB]**
  1 個裝有固定緩衝液的微量離心管**[FB]**
- 1 Eppendorf tube with Substrate Buffer **[SB]**
  1 個裝有反應物緩衝液的微量離心管**[SB]**
- 1 Eppendorf tube with Substrate (X-gluc)**[S]**
  1 個裝有反應物(X-gluc)的微量離心管 **[S]**

Trypanosome suspensions

錐蟲懸浮液

- 1 Eppendorf tube with suspension of Strain 1 **[T1]**

  1 個裝有品系 1 的微量離心管**[T1]**
- 1 Eppendorf tube with suspension of Strain 2 **[T2]**

  1 個裝有品系 2 的微量離心管**[T2]**
- 1 Eppendorf tube with suspension of Strain 3 **[T3]**

  1 個裝有品系 3 的微量離心管**[T3]**

Introduction [2 points]

背景介紹

Trypanosoma brucei is a parasite causing sleeping sickness in humans and nagana in animals. It is transmitted between individuals via the tsetse-fly and is almost exclusively found in Africa south of the Sahara. To better understand the function of different proteins implicated in the life cycle and infection pathway of *T. brucei*, it is the goal to create mutant strains that lack procyclin, but express another protein of interest instead. Procyclin is a surface protein found in *T. brucei* but not in other trypanosome species and is hypothesized to have an effect on the infection pathway. Different trypanosome species rely on different surface proteins for their infectivity. For example, *T. congolense* relies on a surface protein called GARP.

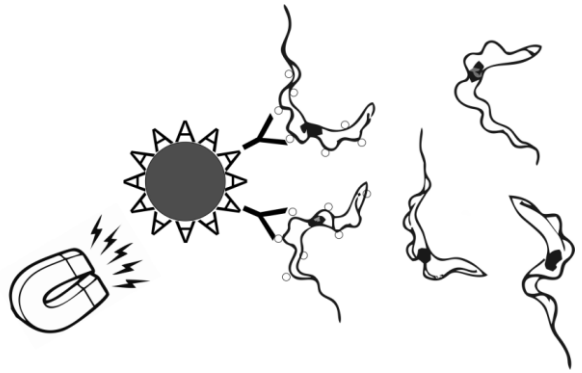
錐蟲 *Trypanosoma brucei* 是人類睡眠病和動物 nagana 病的致病寄生蟲，它藉采采蠅在個體間傳染，且幾乎只分佈於非洲撒哈拉以南地區。不同種類錐蟲的感染力各由不同的表面蛋白決定，例如表面蛋白 procyclin 被認為與感染途徑有關，且只存在 *T. brucei* ，而不存在於其它種之錐蟲；而 *T. congolense* 的感染則依賴另一種表面蛋白 GARP。為了解 procyclin 在 *T. brucei* 生活史和傳染途徑的功能，研究目標為建構一剔除 procyclin 蛋白基因，並表現原本不應存在的 GARP 蛋白基因的 *T. brucei* 突變種。

In this practical you will work with strains of the subspecies *T. brucei brucei*, which can infect domestic and wild animals, but is not dangerous for humans. In a first step, cells were transfected or not with a single construct coding for both GARP and β-glucuronidase and grown as pure strains. β-glucuronidase, which is absent in wild-type *T. brucei*, is a protein that can cleave X-gluc, an artificial substrate, into a blue product that can easily be observed by eye. In this setting, β-glucuronidase serves as a convenient reporter gene that will allow you to recognize the strains carrying a successfully introduced construct by simply incubating the strains with X-gluc (Task 1).

本實作中，使用不同品系的 *T. brucei brucei* (亞種)，這些品系只會感染動物，對人類沒有危險性。首先，這些品系的細胞以一個同時帶有 GARP 和 β-glucuronidase 基因的 DNA 轉殖，β-glucuronidase 原本不存在於 *T. brucei* 中，它能切割 X-gluc 成肉眼可辨識的藍色產物，因此 β-glucuronidase 基因可作為檢定轉殖是否成功的報導基因，只要將這些品系培養於 X-gluc 中即可。

The strains were then subjected to a protocol to delete the gene coding for the protein procyclin. This would allow verifying whether procyclin is indeed important for the infectivity cycle and whether GARP can compensate for the procyclin function. Since the deletion efficiency is not 100%, you will separate the cells where the procyclin gene was successfully deleted from the ones in which the deletion did not work. To achieve this, trypanosome pre-incubated with antibodies specific against procyclin will be separated with magnetic beads coated with protein A that specifically binds to the Fc part of antibodies, as is illustrated below.

接著，這些品系將透過既定的方法將 procyclin 基因剔除，藉此可以探究 procyclin 對感染力的重要性，並可知 GARP 可否取代 procyclin。
由於此既定方法的基因剔除效率並非 100%，你的工作是將已剔除 procyclin 基因的細胞和未成功剔除者分離，為此，將已和能專一性辨識 procyclin 抗體混合的錐蟲，用表面帶有 protein A 的磁珠進行分離。protein A 會專一的吸附抗體的 Fc 部分，實驗原理如下圖所示：



Before starting the practical work, indicate for each of the following statements if it is true or false with a tick (√). **[2 points]**

在開始操作實驗前，先回答以下是非題，以 √ 標示正確 (true)或錯誤(false)

Q1

	true	false
If the suspension of a strain incubated with X-gluc turns blue, the β-glucuronidase gene was successfully introduced. 如果一品系培養於 X-gluc 中呈藍色，表示 β-glucuronidase 基因已轉殖成功		
Inferring the successful introduction of the GARP gene based on the presence of the reporter gene (β-glucuronidase) may result in false positives or negatives if only one gene of the construct was successfully inserted. 如果建構於同一 DNA 上的二個基因在轉殖時，只有單一基因插入染色體，則只藉 β-glucuronidase 基因的存在與否來判定 GARP 基因是否也同時轉入可能會造成誤判		
The location in the genome where the construct is introduced affects the level of gene expression of the introduced genes. 轉殖基因插入染色體的位置會影響此基因的表現量		
A similar approach with magnetic beads and specific antibodies can be used to separate cells, with successful deletion of a gene coding for an intracellular protein, from cells where the deletion did not work. 此一利用磁珠及專一抗體來分離表面蛋白之基因剔除細胞的方法也適用於分離細胞內部蛋白之基因剔除細胞		

Task 1: Presence of β-glucuronidase [12 points]

實作 1：β-glucuronidase 的表現

Part 1.1: Determine the presence of β-glucuronidase [12 points]

1.1：判定 β-glucuronidase 是否表現

Prepare the following reaction mix for each of the three trypanosome strains T1, T2 and T3 in a separate Eppendorf tube and mix by pipetting up and down:

為 3 種錐蟲品系 T1, T2 和 T3 分別製備反應溶液於 3 個微量離心管，在每一個離心管標記：品系編號、你的國家代碼 (如識別名牌所示的 3 個字母)

按下列順序吸取，以微量分注器混合均勻

1. 20 µl of the trypanosome suspension. Since the trypanosomes sink to the bottom of the tube, make sure to mix the tubes by inverting prior to pipetting.

   20 µl 錐蟲懸浮液。因為錐蟲會沉降到管底，確定混合均勻後再吸取

2. 100 µl substrate buffer **(SB)**

   100 µl 反應物緩衝溶液 **(SB)**

3. 10 µl substrate **(S)**

   10 µl 反應物**(S)**

Label each tube with the strain you used as well as with your three letter country code (as indicated on your badge).

為 3 種錐蟲品系 T1, T2 和 T3 分別製備反應溶液於 3 個微量離心管，在每一個離心管標記品系編號和你的國家代碼 (如識別名牌所示的 3 個字母)

按下列順序吸取，以微量分注器混合均勻

1. 20 µl 錐蟲懸浮液。因為錐蟲會沉降到管底，確定混合均勻後再吸取
2. 100 µl 反應物緩衝液 **(SB)**
3. 10 µl 反應物**(S)**

The reaction mixes must be incubated for at least 1 hour at 37°C. Place your flag into the tube on your partition wall to call an assistant who will put your tubes in a water bath. Also, use your flag to indicate to the assistant that you want to get your tubes back from the water bath. Consider on working on the other tasks during the incubation.

將你的協助旗插在你的實驗隔板上，試場助理會來將你的反應管置於水浴中，在 37°C 進行反應至少 1 小時；反應時間結束時，再次將你的協助旗插在你的實驗隔板上，試場助理會將你的反應管取回。在等待水浴時間先做其它題目。

Put your tubes on the yellow sheet with your student code in the corresponding box, they will be photographed and evaluated.

將反應管放在標記有你的學生編號的黃色紙上，置於指定的盒子內，此結果會被拍照並評分

Indicate with a tick (√) for each of the three strains if the sample turned blue or remained colourless after incubation. **[12 points]**

在以下表格內分別勾選(√) 此 3 品系顏色變化的實驗結果

Q2

	Strain T1	**Strain T2**	**Strain T3**
Blue 藍色			
Colourless 無色			

Task 2: Presence of the procyclin protein [86 points]

實作 2：procyclin 蛋白的表現

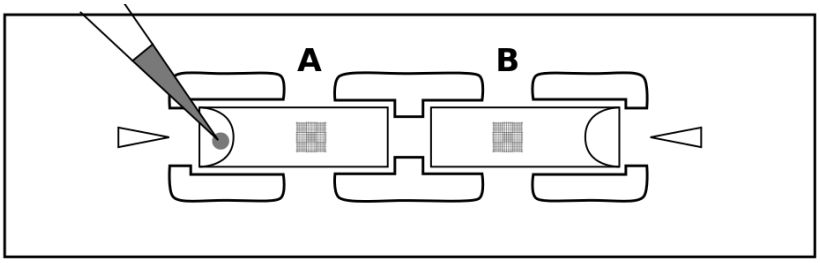
Part 2.1: How to use the counting chamber [1 point]

2.1：如何使用細胞計數槽

You will use counting chambers to determine the density of trypanosomes in parts 2.3 and 2.4. Two counting chambers that can be individually filled are organized on a single slide. **Please be aware that these chambers cannot be cleaned and that no extra counting chambers will be provided. Also these counting chambers do not need a cover slip.**

你要在試題 2.3 和 2.4 使用細胞計數槽去測定錐蟲的密度，每一個計數槽顯微玻片上有 2 個細胞計數槽可獨立使用(如下圖)

注意：此計數槽無法清洗再使用，用完後不會再補發，這種計數槽不需使用蓋玻片



The following steps should be performed when determining the density of trypanosomes in a suspension:

當計算細胞密度時，按以下步驟操作

1. Pipette 10 µl of the suspension into a counting chamber.

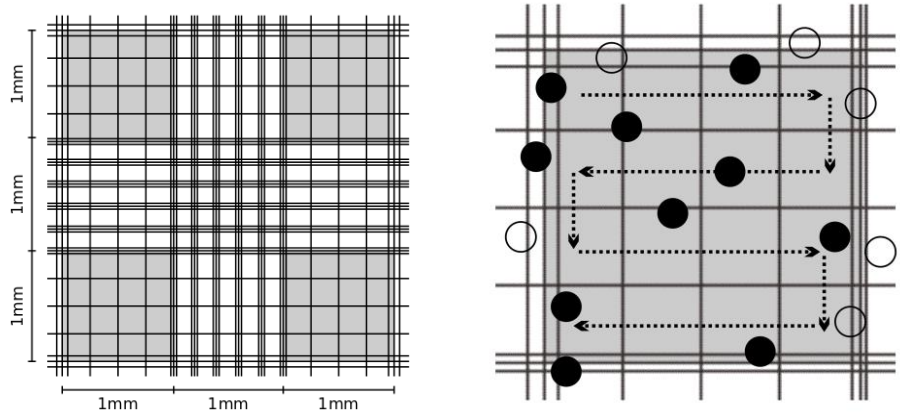
   吸取 10 µl 細胞懸浮液，如上圖加注於計數槽內

2. Wait at least 2 minutes for the cells to sink to the bottom.

   至少等待 2 分鐘，讓細胞沉降於底部

3. Put the slide under the microscope and count the number of trypanosomes individually in three of the four larger squares highlighted in gray.

   將計數槽顯微玻片置於顯微鏡下，在下左圖所示的 4 個灰色方格區塊中選 3 區，分別計算其中所含的錐蟲數目



You may use either the 10x or the 40x objective, whichever you prefer. It is advised to follow a serpentine (snake-like) route to go through each of the smaller squares to avoid losing orientation. In order to prevent a potential bias, count trypanosomes within the square and those crossing the left or bottom limit (filled circles), but not those outside the square or crossing the right or top limit (open circles).

你可以依個人喜好使用 10x 或 40x 物鏡，建議採取蛇行法計數(如上圖右)，依序計算各小格內的細胞數。為避免可能的偏差，只有方格內以及方格左邊界線和下邊界線上的細胞列入計算(如上圖右實心圓)，方格外以及右邊界線和上邊界線上的細胞不列入計算(如上圖右空心圓)

To obtain the trypanosome density from the number of counted trypanosomes, first determine the volume in which the cells are counted and indicate it in the table below. Note that the height of the counting chamber is exactly 0.1 mm and each of counting cell is exactly 1 mm wide (see figure above) **[1 point]**.

計算錐蟲細胞密度，首先要計算每一灰色方格區塊的容積，細胞計數槽的空間高度是 0.1 mm，而每一灰色方格區塊的邊長是 1 mm(如上圖左)，將計算所得容積填入下方表中

Q3

Volume of 1 counting square (mm^3) 單一方格區塊容積	
Volume of 1 counting square (ml) 單一方格區塊容積	

Part 2.2: Washing the magnetic beads

2.2：清洗磁珠

Wash the magnetic beads **(MB)** twice as follows:

依以下步驟(1-3)清洗磁珠 2 次

1. Add 1 ml cold phosphate buffer **(PBSB)** to the tube and mix by pipetting up and down.

   在磁珠管中加入 1 ml 磷酸基緩衝液**(PBSB)**，用微量吸管混合

2. Place the Eppendorf tube in the magnetic holder. Wait at least 1 minute for the magnetic beads to get pulled down.

   將此磁珠離心管放入微量離心管磁力座內，至少等待 1 分鐘讓磁珠落至離心管底部

3. Pipette the supernatant into the liquid waste tube **(LW)**.

   用微量吸管吸取上清液，排棄於液體廢棄物容器

Finally, resuspend the magnetic beads in 35 µl PBSB buffer and put them on ice.

最後，加入 35 µl 磷酸基緩衝液**(PBSB)**，使磁珠懸浮，並放在冰上

Part 2.3: Density of trypanosomes not binding to magnetic beads [37.5 points]

2.3：不被磁珠吸附的錐蟲密度

Pull down trypanosomes expressing procyclin in a sample of each of the three trypanosome strains T1, T2 and T3 as follows:

在品系 T1、T2 和 T3 中，收集表現 procyclin 的錐蟲步驟如下：

1. Pipette 190 µl of the trypanosome suspension in a fresh Eppendorf tube. Mix the trypanosome suspension by inverting prior to pipetting.

   均勻混合錐蟲懸浮液後，吸取 190 µl 懸浮液，放入新的離心管中

2. Add 10 µl of washed magnetic beads. Make sure the beads are resuspended prior to pipetting.

   先均勻混合磁珠懸浮液後，取 10 µl 加入 190 µl 錐蟲懸浮液中

3. Incubate 30 minutes on ice. Resuspend the magnetic beads very gently every 3-5 minutes by inverting and finger-flicking the tube. Consider working on other parts of Task 2 during the incubation.

   放在冰上 30 分鐘，每 3-5 分鐘倒置並輕彈離心管以維持磁珠之懸浮，等候時先做其他部分

4. Pull down the magnetic beads using the magnetic holder.

   時間終了時，用微量離心管磁力座將磁珠吸至管底

5. Transfer the entire supernatant, while the tube is still in the magnetic holder, into a fresh Eppendorf tube and put on ice.

   將離心管留在磁力座上，用微量吸管吸取所有上清液，放入新離心管中，並放在冰上

6. Immediately resuspend the magnetic beads in 50 µl phosphate buffer **(PBSB)** very gently and put on ice.

   吸完立即在此一磁珠離心管中加入 50 µl 磷酸基緩衝溶液**(PBSB)**，溫柔地用微量吸管器讓磁珠懸浮，放在冰上

7. Prepare 100 µl of a 1:10 dilution of the supernatant in phosphate buffer **(PBSB)** in a fresh Eppendorf tube.

   取一新離心管，製備 100 µl 以磷酸基緩衝液**(PBSB)** 作成之 1:10 稀釋的上清液

8. Pipette 36 µl of this dilution into a separate Eppendorf tube and add 4 µl of Fixation Buffer **(FB)**. Mix well by pipetting up and down.

   取 36 µl 的稀釋上清液，加入一新的離心管中，再加入 4 µl 的固定緩衝液 **(FB)**，用微量吸管混合

9. Count the number of trypanosomes according to the protocol in Part 2.1 and enter the values in the table below.

   用 2.1 的方法計算錐蟲數目，將所得結果填入下表中

10. Calculate the mean of the number of trypanosomes per square (precision: 3 positions after the decimal point). You will use these numbers in Part 2.5. [37.5 points]

    計算各品系單一方格區塊的平均錐蟲數目至小數點後第三位，你將在試題 2.5 使用到此平均值

Q4

	Strain T1	Strain T2	Strain T3
Square 1 區塊 1			
Square 2 區塊 2			
Square 3 區塊 3			
Mean counts per square 平均數目(小數點後第三位)			

Part 2.4: Total trypanosome density [25.5 points]

2.4：錐蟲總密度

To determine the total trypanosome density in the original suspension, perform the following steps for each of the three trypanosome strains T1, T2 and T3:

分別計算 T1、T2 和 T3 三品系錐蟲原始的懸浮液總密度，步驟如下：

1. Prepare 100 µl of a 1:10 dilution of the original suspension in phosphate buffer **(PBSB)** in a fresh Eppendorf tube. Mix the trypanosome suspension by inverting prior to pipetting.

   取一新的離心管，製備以磷酸基緩衝液**(PBSB)** 做 1:10 稀釋的原始錐蟲懸浮液 100 µl，注意要先混合均勻再吸取

2. Pipette 36 µl of this dilution into a fresh Eppendorf tube and add 4 µl of Fixation Buffer **(FB)**. Mix well by pipetting up and down.

   取 36 µl 的稀釋原始懸浮液，加入一新的離心管中，再加入 4 µl 的固定緩衝液**(FB)**，用微量吸管混合

3. Count the number of trypanosomes according to the protocol in Part 2.1 and report the values in the table below.

   用 2.1 的方法計算錐蟲數目，將數目填入下表中

4. Calculate the mean and the standard deviation of the number of trypanosomes per square (precision: 3 positions after the decimal point). You will use these numbers in Part 2.5. **[25.5 points]**

   計算各品系的平均錐蟲數目與標準差至小數點後第三位，你將在試題 2.5 中用到此平均值

The formula for the standard deviation (SD) is given below with n being the number of replicates, x_i the value of the replicate and $\bar{x}$ the mean.

標準差(SD)的計算公式如下，n 是重複數，x_i 是各計數值，$\bar{x}$ 是平均值

$$SD = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(x_i - \bar{x})^2}$$

Q5

	Strain T1	Strain T2	Strain T3
Square 1 區塊 1			
Square 2 區塊 2			
Square 3 區塊 3			
Mean counts per square 單一區塊的平均數目			
Standard deviation of counts per square 標準差			

Part 2.5: Success of the deletion of the procyclin gene [9 points]

2.5：procyclin 基因之成功剔除

The ultimate goal is to calculate and compare the density of trypanosomes not binding to magnetic beads to the total trypanosome density in the starting suspension from the average counts observed. However, you will first have to determine the standard error of the mean (SE_{mean}) to decide on the correct number of significant digits. Under the assumption that the counts are normally distributed, the SE_{mean} is given by $SE_{mean} = \frac{SD}{\sqrt{n}}$

Calculate SE_{mean} for the strain for which you observed the largest standard deviation among the counts of trypanosomes per cell from Part 2.4 and enter your result in the table below (precision: 3 position after the decimal point). **[0.6 points]**

最終目標是計算並比較不會吸附於磁珠上的細胞平均密度與原始總細胞平均密度，但首先你要計算平均值標準偏差(SE_{mean})，並決定有效數字的位數。假設計數值是常態分佈，則

$$SE_{mean} = \frac{SD}{\sqrt{n}}$$

自試題 2.4 中，用你所觀測到各品系中最大標準差值來計算平均值標準偏差(SE_{mean})，並將結果填入下表中(準確至小數點後第三位)

Q6

SE_{mean} 平均值標準偏差	

The SE_{mean} tells you the accuracy with which you estimated the mean number of trypanosomes per square. Use this estimate to decide the correct number of significant digits (all digits including the first for which you are uncertain) by comparing the estimated mean plus the SE_{mean} with the estimated mean minus SE_{mean}. For instance, if the mean is 1234.567 and the SE_{mean} 98.765, you will have to compare 1234.567 + 98.765 = 1333.332 with 1234.567 - 98.765 = 1135.802. In this case, there are two significant digits and the mean should be reported as $1.2x10^3$. Indicate the number of significant digits you should use with your data. **[1.5 points]**

平均值標準偏差(SE_{mean})可以告訴你估算平均值的正確度，藉由比較"估算平均值加上 SE_{mean}"與"估算平均值減去 SE_{mean}"，以決定有效數字之位數(含一位不確定值)，

例如：估計平均值是 1234.567，SE_{mean} 是 98.765，你要比較 1234.567 + 98.765 = 1333.332 和 1234.567 - 98.765 = 1135.802，在此例子中，有效數字是二位，平均值應以 $1.2x10^3$ 表示。決定你應採用的有效數字位數，並填於下表中

Q7

Number of significant digits 有效數字位數	

Report in the table below the mean counts per square with and without pull-down for all three strains using the number of significant digits you indicated above. **[0.6 points]**

依照你所決定的有效數字位數，將不會吸附磁珠的細胞平均數目與原始總細胞平均數目重新表示於下表中

Q8

	Strain T1	**Strain T2**	**Strain T3**
Mean counts per square of trypanosomes not binding to magnetic beads (from Part 2.3) 不會吸附磁珠的細胞平均數目 (源自試題 2.3)			
Mean counts per square of total trypanosomes (from Part 2.4) 原始總細胞平均數目 (源自試題 2.4)			

Now use these values to estimate the density of trypanosomes in the dilutions used for counting, and report your values in the table below with the same number of significant digits. **[3.7 points]**

現在使用這些數值去估算稀釋後的細胞密度，以相同位數的有效數字表示於下表中

Q9

	Strain T1	Strain T2	Strain T3
Trypanosomes not binding to magnetic beads /ml in dilution used for counting (from Part 2.3) 不會吸附磁珠的細胞密度 /ml (源自試題 2.3)			
Total trypanosomes / ml in dilution used for counting (from Part 2.4) 原始總細胞平均密度 /ml (源自試題 2.4)			

Finally, calculate both the density of trypanosomes not binding to magnetic beads as well as the total trypanosome density in the original suspension, and report your values in the table below with the same number of significant digits. **[1.1 points]**

最後計算稀釋前的細胞密度，以相同位數的有效數字表示於下表中

Q10

	Strain T1	Strain T2	Strain T3
Trypanosomes not binding to magnetic beads /ml in original suspension (from Part 2.3) 不會吸附磁珠的細胞密度 /ml (源自試題 2.3)			
Total Trypanosomes / ml in original suspension (from Part 2.4) 原始總細胞平均密度 /ml (源自試題 2.4)			

In order to assess the success rate of the gene deletion experiment, calculate the percentage of trypanosomes that did not bind to magnetic beads for each of the three strains. Use the estimates for the densities in the original suspension for your calculations and indicate your results in the table below (precision: only full percentages). **[1.5 points]**

為估算此剔除實驗的成功率，使用稀釋前的原始細胞密度來計算各品系不會吸附於磁珠的細胞比例，以整數百分比表示於下表中

Q11

	Strain T1	Strain T2	Strain T3
Percentage of trypanosomes **not** binding to beads 不會吸附磁珠的細胞百分比			

Part 2.6: Verification of binding to beads [9 points]

2.6：確認吸附於磁珠

You will next verify under the microscope if a reduction in trypanosomes observed after pull down is indeed due to binding of trypanosomes to the magnetic beads. To do so, perform the following steps for each of the three strains:

接下來，你要確認經過磁珠吸附步驟後，細胞數目的減少確是因為錐蟲被磁珠吸附。為達成上述目的，分別對三品系進行下列步驟

1. Pipette 10 µl of the beads, you resuspended in Part 2.3, on a microscope slide.
   自試題 2.3 最後的磁珠懸浮液中吸取 10 µl，滴於載玻片上
2. Cover the drop with a cover slip.
   加上蓋玻片

Make a rough assessment of the fraction of trypanosomes that are attached to a magnetic bead. A good indication that a trypanosome is bound to a bead is when the bead wiggles as the trypanosome moves.

約略估計被磁珠吸附的錐蟲所佔比例，判別錐蟲被磁珠吸附的一個好方法是錐蟲的運動會使磁珠也跟著擺動

In the table below, indicate with a tick (√) for each of the three strains which description best fits your observation. **[9 points]**

在下表中勾選(√)對各品系最適切的描述

Q12

	Strain T1	Strain T2	Strain T3
Practically no unbound trypanosomes in the sample 幾乎沒有不被磁珠吸附的錐蟲			
<50% of the trypanosomes present in the sample are bound <50%的錐蟲被磁珠吸附			
>50% of the trypanosomes present in the sample are bound >50%的錐蟲被磁珠吸附			

Part 2.7: Interpretation of your results [4 points]

2.7：說明你的實驗結果

Indicate with a tick (√) the statement best describing the reduction in trypanosomes in the supernatant after pull down for each strain. **[3 points]**

在下表中勾選(√)最能說明上清液中錐蟲數目減少的敘述

Q13

	Strain T1	Strain T2	Strain T3
A reduction, at least in part due to binding to beads 錐蟲數目的減少，至少有部分原因是因為被磁珠吸附			
No or only a purely stochastic change 沒有改變或只是隨機結果			

Indicate with a tick (√) the strain where the deletion was most efficient. **[1 point]**

在下表中勾選(√)剔除最有效的品系

Q14

	Strain T1	Strain T2	Strain T3
Highest deletion efficiency 剔除效率最高的品系			

End of the Practical Exam

本實作考試完成