18th INTERNATIONAL BIOLOGY OLYMPIAD

July 15 – 22, 2007

第 18 屆國際生物奧林匹亞

PRACTICAL EXAM 4 實作 4

GENETICS 遺傳學

【會先進行色盲檢驗】

TASK A. Sequence confirmation of a cDNA 23 marks

一段 cDNA 序列的組成 (20 分)

~~TASK B. Genetics of coat colour in dogs 16 marks~~

TASK C. Genetic control of seed coat colour and seed shape in beans 20 marks

豆子種皮顏色及種子性狀遺傳的控制 (20 分)

Time allowed: 90 minutes

共 90 分鐘

WRITE ALL ANSWERS IN THIS EXAM BOOKLET.

所有答案要寫在這本試卷上

WRITE YOUR 4-DIGIT STUDENT CODE IN THE BOX BELOW AND ON THE TOP OF EACH PAGE OF THIS BOOKLET

將你的 4 碼學生代碼寫在下欄中並寫在其他各頁的上方

Student code: 學生代碼	

TASK A. Sequence Confirmation of a cDNA (23 marks)
一段 cDNA 序列的組成 (23 分)

Objective: To isolate plasmid DNA containing a cDNA of interest and to determine the sequence of the cDNA**.**

目的：分離一段含有待研究之 cDNA 的質體 DNA，並決定此 cDNA 的序列

Introduction:
To over-express a gene of interest in a plant or animal you must first isolate the gene of interest in the form of a cDNA. You have done this and in order to amplify this DNA, you have cloned it into the pBluescript SK plasmid vector which you have subsequently used to transform bacteria cells. You must now carry out a quick plasmid preparation to isolate the plasmid and confirm the sequence of your cDNA insert.
介紹：要過度表現植物或動物有待研究的一個基因，你必須先以 cDNA 的形式分離出這段基因；若你已完成分離，要放大這段 DNA 並 clone 在 pBluescript SK 質體的載體中，接著用這載體來轉形細菌；你必須快速進行質體製備，以分離質體並確定此 cDNA 插入段的序列。

Materials 材料	**Quantity (數)量**
➢ Bacterial cell culture 細菌培養液	4 mL
➢ 1.5 mL microcentrifuge tubes 1.5 mL 微離心管	5
➢ Microcentrifuge rack 微離心管架	1
➢ P1000 micropipettor P1000 微吸管	1
➢ Box of 200-1000 uL pipette tips 200-1000 uL 吸管尖盒	1
➢ GET buffer (1.5 mL tube) GET 緩衝液(1.5 mL 小離心管)	0.5 mL
➢ 10% Sodium Dodecyl Sulphate (1.5 mL tube) 10% SDS (1.5 mL 小離心管)	0.5 mL
➢ 2 N NaOH (1.5 mL tube) 2 N NaOH (1.5 mL 小離心管)	0.5 mL
➢ 3 M Potassium 5 M Acetate (1.5 mL tube)	0.5 mL

3 M 鉀 5 M 醋酸 (1.5 mL 小離心管)

- 95% ethanol (Falcon tube) 3 mL
  95% 乙醇 (試管)
- Distilled water (Falcon tube) 3 mL
  蒸餾水 (試管)
- Timer 1
  計時器
- Tube labels 2
  試管標籤
- Marker pen 1
  簽字筆
- Red card 1
  紅卡片
- Garbage (tips & tubes) bag 1
  垃圾袋
- Access to a microcentrifuge
  微離心機
- Access to vortex
  震盪機

NOTE: Before beginning this task, be sure that you have all the materials listed above. If you do not, raise your RED card to call a lab assistant.

注意：開始動手前，先確定你有上述所有材料，若有缺少，舉起紅卡片請監考人員來幫忙

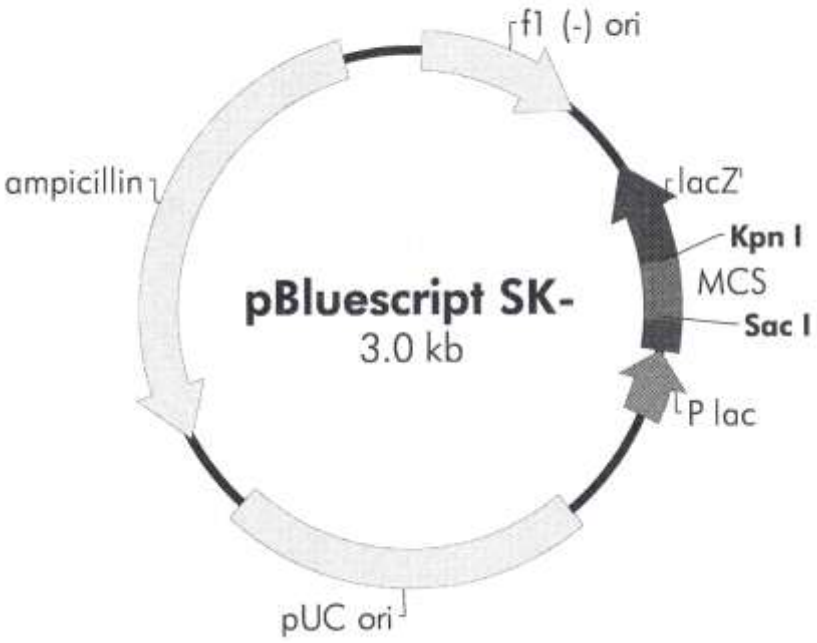
Procedure 步驟

1. Pipette 1.5 mL of bacterial culture into each of two 1.5 mL microcentrifuge tubes.
   兩支 1.5 mL 微離心管中各加入 1.5 mL 細菌培養液
2. Centrifuge the tubes in a benchtop microcentrifuge for 1 minute - make sure that the centrifuge rotor is **BALANCED**.
   用微離心機離心 1 分鐘，離心前要確定離心轉子(rotor)的平衡
3. Completely remove and discard back into the overnight tube the growth medium from each tube.
   將兩管中的上清液完全移除

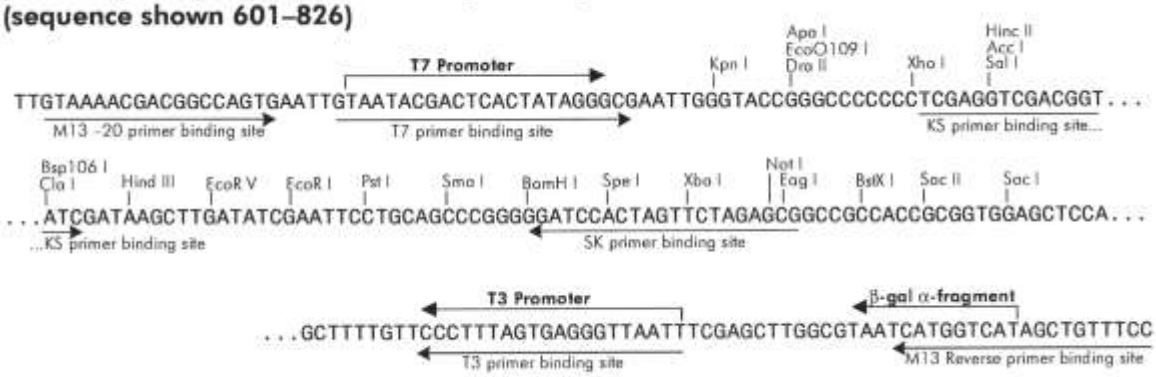
4. Add 100 uL of GET (Glucose-EDTA-Tris) buffer pH 7.9 to the cell pellet (no need to cap the tubes) - vortex vigorously to resuspend the pellet and leave at room temperature for 5 minutes.
   在細胞沈澱上加入 100 uL pH 7.9 的 GET (Glucose-EDTA-Tris)緩衝液(不需蓋上蓋子)，激烈震盪 5 分鐘以懸浮細胞的沈澱，置於室溫 5 分鐘
5. In a separate 1.5 mL microcentrifuge tube, make a combined mixture of 1% SDS and 0.2 N NaOH in water to a final volume of 1 mL.
   在另一支 1.5 mL 微離心管中，混合 1% SDS 及 0.2 N NaOH 到水中，使最後體積成為 1 mL.
6. To each tube from 4. above add 200 uL of this freshly prepared mixture of 1% SDS and 0.2 N NaOH - cap the tubes and invert 4-5 times.
   在上述步驟 4 的每支 1.5 mL 微離心管中，加入新配的 200 uL1% SDS 及 0.2 N NaOH 混合液，蓋上蓋子並倒置、正置 4-5 次
7. Incubate at room temperature for 3 minutes.
   在室溫培養 3 分鐘
8. To each tube add 150 uL 5M KOAc (3 M potassium and 5 M acetate), cap the tubes and shake briefly by hand to mix.
   在每支小管中加入 150 uL 5M KOAc (3 M 鉀與 5 M 醋酸) ，蓋上蓋子用手搖混合
9. Incubate at room temperature for 3 minutes.
   在室溫培養 3 分鐘
10. Centrifuge the tubes for 3 minutes - full speed in microcentrifuge - **remember to balance the rotor**.
    用微離心機全速離心 3 分鐘 – <u>記得離心前要先平衡</u>
11. Label 2 clean microcentrifuge tubes with your 4-digit student code number.
    將你的 4 碼學生代碼標記在乾淨的 2 支微離心管上
12. Pipette the supernatant from each of the centrifuged tubes into each of the clean tubes. Discard the **original** tube which now contains a white pellet - this is bacterial chromosomal DNA.
    將每支微離心管中的上清液吸出，置於新的微離心管中，棄置<u>原</u>管，該管中的白色沈澱即為細菌染色體 DNA
13. Add 800 uL of 95% ethanol to each tube. Cap the tubes, shake vigorously by hand for 10 sec and leave on the bench for 10 minutes.
    在各管中加入 800 uL 95%乙醇，蓋上蓋子用手搖混合 10 秒，靜置 10 分鐘
14. Centrifuge the tubes for 5 minutes - full speed in microcentrifuge.
    用微離心機全速離心 5 分鐘
15. Pour off the supernatant from each tube, cap the tube and **raise your RED card**.
    倒出各管中的上清液，蓋上蓋子並**舉起你的紅卡片**
16. The lab assistant will check your pellet (10 marks for a white pellet).
    讓監考人員檢查你的沈澱 (白色沈澱得 10 分)
17. The lab assistant will then give you the sequence trace for your plasmid and cDNA. The cDNA was sequenced from the T_3 promoter.
    監考人員會給你質體及 cDNA 序列標記，此 cDNA 將由 T_3 的起動子開始定序
18. Check your sequence (starting at nucleotide 21) against that for the pBluescript vector and answer the questions on page 5.
    以 pBluescript 載體(由第 21 核苷酸起)檢視你的序列，並回答第 5 頁的問題

PLASMID MAP AND MULTIPLE CLONING SITE SEQUENCE FOR pBLUESCRIPT

pBLUESCRIPT 的質體輿圖及多選殖位(multiple cloning site)序列



pBluescript SK (+/–) Multiple Cloning Site Region
(sequence shown 601–826)



Questions (13 marks) **問題** (13 分)

1. The enzyme site into which you cloned your fragment of DNA is _____.
   用以選殖你 DNA 片段序列的酵素切位為何
   NOTE: The first letter of the enzyme's name is located above the first nucleotide of its recognition sequence. (5 marks).
   注意：酵素名稱的第一字母是在其所辨識序列的第一個核苷酸上面 (5 分)

2. List the first 20 nucleotides of your fragment of DNA (not including the restriction site sequence). (2 marks)
   寫出你那段 DNA 最前面的 20 個核苷酸的序列 (不包括限制位的序列) (2 分)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Nucleotide 核苷酸																				

3. Find the first start codon. Using the genetic code table provided, and starting with the start codon, translate the first 21 nucleotides into their appropriate amino acids. (4 marks)
   找出第一個起始密碼子，用所提供的遺傳密碼表，由起始密碼子開始將頭 21 個核苷酸譯成對應的氨基酸序列 (4 分)

Start codon

Amino acid 氨基酸																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
nucleotide 核苷酸																					

4. (a) If the nucleotide at position 13 was mutated to an 'A', what would be the corresponding amino acid? (1 mark)
   如果核苷酸第 13 個位置突變成為 "A"，將會對應成為何種氨基酸 (1 分)

   (b). If the nucleotide at position 14 was mutated to an 'A', what would be the corresponding amino acid? (1 mark)
   如果核苷酸第 14 個位置突變成為 "A"，將會對應成為何種胺基酸 (1 分)

GENETIC CODE TABLE 遺傳密碼表

This table shows the 64 codons and the amino acid each codon codes for. The direction is 5' to 3'.

本表顯示 64 個密碼子及其所對應的氨基酸，方向是 5' to 3'

		2nd base			
		U	**C**	**A**	**G**
1st base	**U**	UUU (Phe/F)Phenylalanine	UCU (Ser/S)Serine	UAU (Tyr/Y)Tyrosine	UGU (Cys/C)Cysteine
		UUC (Phe/F)Phenylalanine	UCC (Ser/S)Serine	UAC (Tyr/Y)Tyrosine	UGC (Cys/C)Cysteine
		UUA (Leu/L)Leucine	UCA (Ser/S)Serine	UAA Ochre (*Stop*)	UGA Opal (*Stop*)
		UUG (Leu/L)Leucine	UCG (Ser/S)Serine	UAG Amber (*Stop*)	UGG (Trp/W)Tryptophan
	C	CUU (Leu/L)Leucine	CCU (Pro/P)Proline	CAU (His/H)Histidine	CGU (Arg/R)Arginine
		CUC (Leu/L)Leucine	CCC (Pro/P)Proline	CAC (His/H)Histidine	CGC (Arg/R)Arginine
		CUA (Leu/L)Leucine	CCA (Pro/P)Proline	CAA (Gln/Q)Glutamine	CGA (Arg/R)Arginine
		CUG (Leu/L)Leucine	CCG (Pro/P)Proline	CAG (Gln/Q)Glutamine	CGG (Arg/R)Arginine
	A	AUU (Ile/I)Isoleucine	ACU (Thr/T)Threonine	AAU (Asn/N)Asparagine	AGU (Ser/S)Serine
		AUC (Ile/I)Isoleucine	ACC (Thr/T)Threonine	AAC (Asn/N)Asparagine	AGC (Ser/S)Serine
		AUA (Ile/I)Isoleucine	ACA (Thr/T)Threonine	AAA (Lys/K)Lysine	AGA (Arg/R)Arginine
		AUG (Met/M)Methionine	ACG (Thr/T)Threonine	AAG (Lys/K)Lysine	AGG (Arg/R)Arginine
	G	GUU (Val/V)Valine	GCU (Ala/A)Alanine	GAU (Asp/D)Aspartic acid	GGU (Gly/G)Glycine
		GUC (Val/V)Valine	GCC (Ala/A)Alanine	GAC (Asp/D)Aspartic acid	GGC (Gly/G)Glycine
		GUA (Val/V)Valine	GCA (Ala/A)Alanine	GAA (Glu/E)Glutamic acid	GGA (Gly/G)Glycine
		GUG (Val/V)Valine	GCG (Ala/A)Alanine	GAG (Glu/E)Glutamic acid	GGG (Gly/G)Glycine

Task B. 刪除

Task C. Genetic Control of Seed Coat Colour and Seed Shape in Beans (20 points)

豆子種皮顏色及種子性狀遺傳的控制 **(20 分)**

Material 材料

- 1 plastic bag containing flat red parent beans – Do not open
  1 塑膠袋裝有扁平紅色的親代豆子 **– 不可打開**
- 1 plastic bag containing round red parent beans – Do not open
  1 塑膠袋裝有圓厚紅色的親代豆子 **– 不可打開**
- 1 plastic bag containing F_1 seeds (flat yellow) from the cross between the parent beans – Do not open
  1 塑膠袋裝有上述親代交配後所產生的第一子代豆子(扁平黃色) **– 不可打開**
- 1 plastic bag of F_2 bean seeds (representing 250 F_2 plants) – This bag may be opened
  1 塑膠袋裝有用以代表 250 個第二子代植株每株一顆的豆子 **– 此袋可以打開**

To help you answer the questions below, fill in the following table:
為了幫助你回答下列問題，請填寫下列空格

Generation 世代	**Seed shape** **種子形狀** (round or flat) （圓厚或扁平）	**Seed coat colour** **種皮顏色** (yellow or red) （黃色或紅色）
Parent 1 （親代 1）		
Parent 2 （親代 2）		
F_1 from a cross between these two parents （第一子代，來自於上述親代互交）		

Answer the following questions.
回答下列問題

1. Is the seed coat colour controlled by (circle one)
   種皮顏色受到何種方式調控（圈選一個答案）
   (i) one gene
   單一基因
   (ii) more than one gene? (1 mark)
   超過一個基因 (1 分)

2. a) Red seed coat colour is (circle one)
紅色種皮是何種性狀（圈選一個答案）

(i) dominant
顯性
(ii) partially dominant
部份顯性
(iii) recessive (1 mark)
隱性 (1 分)

b) Round seed shape is (circle one)
圓厚種子是何種性狀（圈選一個答案）

(i) dominant
顯性
(ii) partially dominant
部份顯性
(iii) recessive (1 mark)
隱性 (1 分)

3. (a) There are four phenotypes in your sample of F_2 seeds. Classify the seeds into these phenotypic classes and write the number of each phenotype in the table below. (2 marks)
F_2 種子有四種外表型，將這些種子依外表型分開並將每種的數目寫在下表中 (2 分)

Phenotype 外表型 **(seed colour/ seed shape)** **(種子顏色/種子形狀)**	**Number of seeds 種子數** **(= number of F_2 plants)** **(= F_2 植株數目)**
round, red 圓厚紅色	
flat, red 扁平紅色	
round, yellow 圓厚黃色	
flat, yellow 扁平黃色	
Total 總數	

Use these F2 segregation data to answer the following questions:
用這些 F_2 分離的資料回答下列問題：

4. (a) From your data how many genes could be controlling seed shape? _____ (1 mark)
從你的資料來看，多少基因可能控制種子形狀？ (1 分)

(b) How many round beans and how many flat ones would you expect in a population this size?
在這樣大小的族群中，你期待會有多少圓厚的豆子？多少扁平的豆子？

ROUND _____ FLAT _____ (2 marks)
圓厚 扁平 (2 分)

(c) Is this segregation ratio significantly different from the observed ratio (circle one)?
這分離的比率與觀察到的比率差別是否有意義 （圈選一個答案）

YES **NO** (1 mark)
是 否 (1 分)

And what is the probability? (3 marks)
機率是多少？ _____ (3 分)

5. (a) From your data how many genes could be controlling seed coat color? _____ (1 mark)
從你的資料來看，多少基因可控制種皮的顏色？ (1 分)

(b) How many red beans and how many yellow beans would you expect in a population this size?
在這樣大小的族群中，你期待會有多少紅色的豆子？多少黃色的豆子？

RED _____ YELLOW _____ (3 marks)
紅色 黃色 (3 分)

(c) Is this segregation ratio significantly different from the observed ratio? (circle one)
這分離的比率與觀察到的比率差別是否有意義 （圈選一個答案）

YES **NO** (1 mark)
是 否 (1 分)

And what is the probability? (3 marks)
機率是多少？ _____ (3 分)

Chi-square Distribution 卡方分佈

	Probability 機率										
df	0.95	0.90	0.80	0.70	0.50	0.30	0.20	0.10	0.05	0.01	0.001
1	0.004	0.02	0.06	0.15	0.46	1.07	1.64	2.71	3.84	6.64	10.83
2	0.10	0.21	0.45	0.71	1.39	2.41	3.22	4.60	5.99	9.21	13.82
3	0.35	0.58	1.01	1.42	2.37	3.66	4.64	6.25	7.82	11.34	16.27
4	0.71	1.06	1.65	2.20	3.36	4.88	5.99	7.78	9.49	13.28	18.47

- THE END –

檢查各頁上方是否已寫好 **4** 碼學生代碼

HAVE YOU WRITTEN YOUR 4-DIGIT STUDENT CODE ON THE TOP OF EACH PAGE?