18th INTERNATIONAL BIOLOGY OLYMPIAD

July 15 – 22, 2007

PRACTICAL EXAM 4
GENETICS

TASK A. Sequence confirmation of a cDNA 23 marks

~~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

Time allowed: 90 minutes

WRITE ALL ANSWERS IN THIS EXAM BOOKLET.

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

Student code:	

TASK A. Sequence Confirmation of a cDNA (23 marks)

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

Introduction:
A cDNA gene has been inserted into the MCS of the pBluescript SK plasmid vector. This plasmid has been transformed and amplified in bacterial cells. To confirm the presence of the cDNA insert you must carry out the following procedure to isolate the plasmid DNA. This purified plasmid DNA can then be sequenced to determine the presence of the insert.

Materials	Quantity
➢ Bacterial cell culture	4 mL
➢ 1.5 mL microcentrifuge tubes	5
➢ Microcentrifuge tube rack	1
➢ P1000 micropipettor	1
➢ Box of 200-1000 uL pipette tips	1
➢ Glucose-EDTA-Tris buffer / GET (1.5 mL tube)	0.5 mL
➢ 10% Sodium Dodecyl Sulphate /SDS (1.5 mL tube)	0.5 mL
➢ 2 N NaOH (1.5 mL tube)	0.5 mL
➢ 3 M Potassium 5 M Acetate (1.5 mL tube)	0.5 mL
➢ 95% ethanol (Falcon / 50mL tube)	3 mL
➢ Distilled water (Falcon / 50mL tube)	3 mL
➢ Timer	1
➢ Tube labels	2
➢ Marker pen	1
➢ Red card	1
➢ Garbage bag for tips & tubes	1
➢ Access to a microcentrifuge	
➢ Access to vortex	

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.

Procedure

1. Pipette 1.5 mL of bacterial culture into each of two 1.5 mL microcentrifuge tubes.

2. Centrifuge the tubes in a benchtop microcentrifuge for 1 minute - make sure that the centrifuge rotor is **BALANCED**.

3. Completely remove and discard back into the overnight tube the growth medium from each tube.

4. Add 100 µL 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.

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.

6. To each tube from 4. above add 200 µL of this freshly prepared mixture of 1% SDS and 0.2 N NaOH - cap the tubes and invert 4-5 times.

7. Incubate at room temperature for 3 minutes.

8. To each tube add 150 µL 5M KOAc (3 M potassium and 5 M acetate), cap the tubes and shake briefly by hand to mix.

9. Incubate at room temperature for 3 minutes.

10. Centrifuge the tubes for 3 minutes - full speed in microcentrifuge - **remember to balance the rotor**.

11. Label 2 clean microcentrifuge tubes with your 4-digit student code number.

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.

13. Add 800 µL of 95% ethanol to each tube. Cap the tubes, shake vigorously by hand for 10 sec and leave on the bench for 10 minutes.

14. Centrifuge the tubes for 5 minutes - full speed in microcentrifuge.

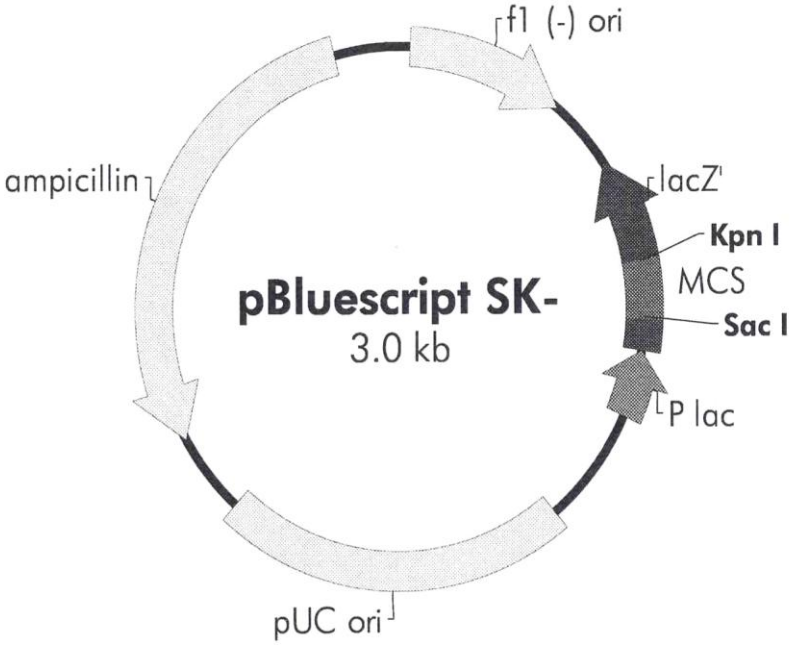
15. Pour off the supernatant from each tube, cap the tube and **raise your RED card**.

16. The lab assistant will check your pellet (10 marks for a white pellet).

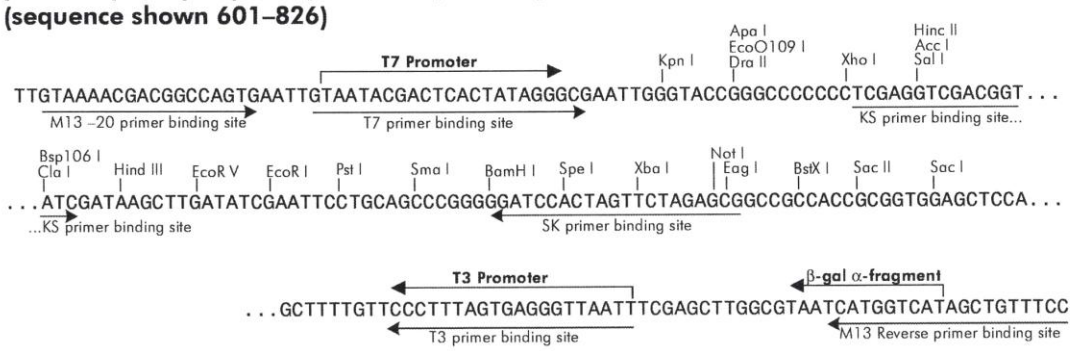
17. The lab assistant will then give you the sequence trace for your plasmid and cDNA. The cDNA was sequenced from the T_7 promoter.

18. Check your sequence (starting at nucleotide 21) against that for the pBluescript vector and answer the questions on page 5.

PLASMID MAP AND MULTIPLE CLONING SITE SEQUENCE FOR pBLUESCRIPT



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



Questions (13 marks)

1. The enzyme site into which you cloned your fragment of DNA is _____.

NOTE: The first letter of the enzyme's name is located above the first nucleotide of its recognition sequence. (5 marks).

2. Give the first 20 bases of your fragment of DNA (not including the restriction site sequence). (2 marks)

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

3. Find the start codon. Using the genetic code table provided on page 6, and starting with the start codon, translate the first 21 bases into their appropriate amino acids. (4 marks)

Start codon

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

4. (a) If the base at position 13 was mutated to an 'A', what would be the corresponding amino acid? (1 mark)

(b). If the base at position 14 was mutated to an 'A', what would be the translated amino acid? (1 mark)

GENETIC CODE TABLE

This table shows the 64 codons and the amino acid each codon codes for. The direction is 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. Genetics of Dog Coat colour (16 marks)

Materials

➔ coloured photograph of four breeds of dog

Procedure

1. Examine the colour photographs of the four dogs. The **dominance relationships** for coat colour is, from left to right,

K – solid black; **k** – hair shaft changes colour (agouti)
E – Wildtype; **e** – red
MM – White; **Mm** – Merle (intermingling of white hair with coloured hair) ;
mm – coloured
$\mathbf{a^w}$ – hair shaft changes colour 3 times; $\mathbf{a^y}$ – Sable (hair red with black tip);
$\mathbf{a^t}$ – black and tan; **a** – black

2. The **genotypes** of the dogs shown in the colour photographs are

Shetland Sheepdog = k/k, E/E, a/a, M/m
Australian Shepherd = k/k, E/E, a^t/a^t, M/m
German Shepherd Dog = k/k, E/E, a/a, m/m
Tervuren = k/k, E/E, a^y/a^y, m/m

3. Using the dogs shown in the coloured photographs, determine **ALL** possible genotypes and phenotypes of puppies from the matings listed in the table on the page 8.

(2 marks per mating genotype and 2 marks per mating phenotype = 16 marks)

4. Write your answers in the appropriate column of the following table:

Mating	**Genotype**	**Phenotype**
Shetland Sheepdog x Australian Shepherd		
Shetland Sheepdog x German Shepherd		
German Shepherd x Tervuren		
Tervuren x Australian Sheepdog		

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

Material

- 1 plastic bag containing flat red parent beans **– DO NOT OPEN**
- 1 plastic bag containing round red parent beans **– DO NOT OPEN**
- 1 plastic bag containing F_1 seeds (flat yellow) from the cross between the parent beans **– DO NOT OPEN**
- 1 plastic bag of F_2 bean seed representing 250 F_2 plants **– MAY OPEN THIS BAG**

To help you answer the questions below, fill in the following table:

Generation	**Seed shape** (round or flat)	**Seed coat colour** (yellow or red)
Parent 1		
Parent 2		
F_1 from a cross between these two parents		

Answer the following questions.

1. Is the seed coat colour controlled by (circle one). (1 mark)

   (i) one gene

   (ii) more than one gene?

2. a) Red seed coat colour is (circle one). (1 mark)

   (i) dominant

   (ii) partially dominant

   (iii) recessive

b) Round seed shape is (circle one). (1 mark)

(i) dominant

(ii) partially dominant

(iii) recessive

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)

Phenotype (seed colour/ seed shape)	**Number of seeds (= number of F_2 plants)**
round, red	
flat, red	
round, yellow	
flat, yellow	
Total	

Use these F2 segregation data to answer the following questions:

4. (a) From your data how many genes could be controlling seed shape? _____ (1 mark)

(b) How many round beans and how many flat ones would you expect in a population this size?

ROUND _____ FLAT _____ (2 marks)

(c) Is this segregation ratio significantly different from the observed ratio (circle one)?

YES **NO** (1 marks)

And what is the probability? _____ (3 marks)

5. (a) From your data how many genes could be controlling red seed coat colour?
_____ (1 mark)

(b) How many red beans and how many yellow beans would you expect in a population this size?

RED _____ YELLOW _____ (3 marks)

(c) Is this segregation ratio significantly different from the observed ratio? (circle one)

YES **NO** (1 marks)

And what is the probability? _____ (3 marks)

Chi-square Distribution

	Probability										
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 –

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