ENGINEERING TRIPOS PART IIA

Thursday 5 May 2011 2.30 to 4

Module 3G1

INTRODUCTION TO BIOSCIENCE

*Answer not more than **three** questions.*

All questions carry the same number of marks.

*The **approximate** percentage of marks allocated to each part of a question is indicated in the right margin.*

Answers to questions in each section should be tied together and handed in separately.

There are no attachments

STATIONERY REQUIREMENTS
Single-sided script paper

SPECIAL REQUIREMENTS
CUED approved calculator allowed

You may not start to read the questions printed on the subsequent pages of this question paper until instructed that you may do so by the Invigilator

Final Version

1 (a) What reagents are needed to carry out a polymerase chain reaction? [20%]

(b) PCR can be used to fuse different DNA fragments together. Explain why it cannot be used to directly build megabase-scale DNA constructs. [20%]

(c) A PCR reaction is set up to amplify a human exon from a blood sample. When the products of the PCR reaction are separated by agarose gel electrophoresis two bands are seen: one is the expected size and the other is slightly smaller. Provide a possible explanation for this result. [25%]

(d) A PCR reaction is set up but inadvertently the researcher adds one hundredth of the correct amount of one of the primers. Describe the course of the subsequent reaction and the most likely dominant product that will be generated. [35%]

2 (a) What are the three key components of evolution by natural selection? [20%]

(b) In a continuous liquid culture, fresh growth medium continually displaces cell-containing medium such that the overall volume remains constant and the cell density can be controlled. Suppose two independent mutations arise in a large ($\sim 10^{12}$ cells) population of bacterial cells grown in continuous liquid culture, seeded from one colony. Both mutations allow the cells containing them to grow faster but the effect is significantly stronger in mutation 'A' than in mutation 'B'. How do you expect the frequency of each mutation within the population to change over time? [20%]

(c) In the same population as in part (b), a mutation arises that does not confer any growth advantage or disadvantage. How do you expect the frequency of this mutation to vary with time? [25%]

(d) A cell-surface protein similar to an antibody binds gold with low affinity. A researcher would like to evolve variants that bind gold more strongly with a view to purifying gold from sea water. Outline a directed evolution strategy to achieve this. [35%]

3 (a) Metabolic networks are responsible for the conversion of building blocks (e.g. sugars, amino acids, nucleotides) into macromolecules (e.g. polysaccharides, proteins, DNA, RNA). How is this achieved within cells? [20%]

(b) In the brewing process the feedstock wort (containing maltose as main carbon source) is converted to ethanol by *Saccharomyces* yeasts. Show the net result of catabolism of maltose (a disaccharide containing two molecules of glucose) by alcoholic fermentation. [20%]

(c) Briefly define metabolic engineering, and its main goal and principal strategies. [30%]

(d) Metabolic engineering efforts, which have tried to maximize the synthesis of a particular product (e.g. aromatic amino acids) by engineering a single rate-limiting step, have proved unsuccessful. Explain the reason for this and why successful examples involve the simultaneous manipulation of a group of enzymes. [30%]

(TURN OVER

4 (a) A genetic engineer wants to express the peptide encoded by the DNA sequence below. Referring to the codon table supplied in Figure 1 (over page), what amino-acid sequence is encoded in the DNA sequence below? Assume that the first codon starts at the first base.

GCCCUUAUCGGCAAUGACAACGCG [20%]

(b) How would the above sequence need to be adapted so that the peptide is properly translated? [25%]

(c) The researcher wishes to maximise expression of the peptide in malaria, the codon usage table of which is given in Fig. 1. Write down the DNA sequence most likely to result in high expression given the information available. [25%]

(d) (i) Outline what factors, other than codon usage, could affect protein expression yield. [15%]

(ii) A different codon optimised recombinant DNA construct was expressed in *Bacillus*. The protein was produced, but at a lower level than an identical non-optimised construct. What is the most likely explanation for this? [15%]

(cont.

Fig. 1: *Plasmodium vivax* codon table showing codon usage

Columns:

Codon; single letter amino-acid code; frequency of codon usage

UUU	F	22.6	UCU	S	9.1	UAU	Y	15.1	UGU	C	9.9
UUC	F	17.0	UCC	S	13.7	UAC	Y	26.8	UGC	C	10.5
UUA	L	13.7	UCA	S	9.2	UAA	*	0.9	UGA	*	0.2
UUG	L	18.9	UCG	S	8.2	UAG	*	0.3	UGG	W	4.9
CUU	L	9.0	CCU	P	5.7	CAU	H	6.9	CGU	R	1.5
CUC	L	14.0	CCC	P	9.4	CAC	H	10.2	CGC	R	2.7
CUA	L	10.0	CCA	P	13.4	CAA	Q	20.6	CGA	R	2.3
CUG	L	17.4	CCG	P	4.1	CAG	Q	14.8	CGG	R	1.4
AUU	I	22.7	ACU	T	11.8	AAU	N	34.8	AGU	S	12.7
AUC	I	16.9	ACC	T	15.4	AAC	N	32.7	AGC	S	17.8
AUA	I	16.5	ACA	T	14.3	AAA	K	53.7	AGA	R	10.9
AUG	M	19.6	ACG	T	11.1	AAG	K	51.3	AGG	R	8.6
GUU	V	12.8	GCU	A	11.7	GAU	D	28.6	GGU	G	10.9
GUC	V	10.0	GCC	A	16.3	GAC	D	26.2	GGC	G	12.4
GUA	V	15.0	GCA	A	26.6	GAA	E	54.1	GGA	G	22.4
GUG	V	19.7	GCG	A	13.3	GAG	E	34.4	GGG	G	10.6

END OF PAPER