Version: Final

EGT2
ENGINEERING TRIPOS PART IIA

Friday 24 April 2015 9.30 to 11.00

Module 3G1

INTRODUCTION TO MOLECULAR BIOENGINEERING

*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.*

Write your candidate number not your name on the coversheet.

STATIONERY REQUIREMENTS
Single-sided script paper

SPECIAL REQUIREMENTS TO BE SUPPLIED FOR THIS EXAM
CUED approved calculator allowed

10 Minutes reading time is allowed for this paper

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

Version: Final

1 (a) (i) Explain how the polymerase chain reaction (PCR) works, by means of diagrams that show the first three cycles. [30%]

(ii) Assuming a typical PCR reaction with 30 cycles, comment on the amplification rates of the different classes of molecules described in (i). [20%]

(b) Different strains of the same species of plant can produce flowers that are white, pink or red, depending on whether they carry 0, 1 or 2 functional copies respectively of a gene that encodes a pigment-generating enzyme.

PCR primers are designed that can amplify this gene, generating a product of 1000 bases in size. When these primers are used to amplify DNA from a white-flowering strain, a longer product of 1300 bases is observed.

(i) Give a possible molecular explanation for the lack of pigmentation in the white-flowering strain. [10%]

(ii) A pink-flowering and a white-flowering strain are crossed. DNA is prepared from their progeny, and PCR reactions carried out with the above PCR primers. Outline a typical technique used to assess the size of the PCR products, and explain the results expected for white, pink and red flowers. [20%]

(iii) What is the expected ratio of white, pink and red flowers among the progeny and why? [20%]

2 Antibodies are key molecules in the adaptive immune system.

(a) Sketch the structure of the basic antibody molecule, labelling the key parts. [10%]

(b) Briefly explain how it is that the antibody molecule diversity observed in an individual is far higher than the number of protein-coding genes in that individual's genome. [30%]

(c) Scientists have made gene fusions between antibody genes and bacteriophage coat protein genes, which result in the antibody fusion protein being expressed on the surface of the bacteriophage. By doing this on a large scale, with many different antibody genes, one can generate a high diversity library. This allows screening to be carried out, for instance by immobilising some target antigen of interest on beads, allowing the bacteriophage library to bind the beads, and then washing away unbound/weakly bound bacteriophage. Tightly bound bacteriophage can then be eluted and used to infect bacteria, allowing generation of further bacteriophage with the same binding specificities.

(i) Outline the steps needed to isolate the antibody genes present in a rat in order to make the above library. [10%]

(ii) What key issue would you consider when designing the fusion between the antibody gene and the coat protein gene? [10%]

(iii) Briefly outline the approach you might take to fuse the material generated in (i), which contains many antibody genes, with bacteriophage vector molecules, in order to generate an antibody fusion library. [20%]

(iv) The resulting library is screened with a bead-immobilised antigen and the tightly bound bacteriophage particles eluted and used to infect further host bacteria in order to produce more bacteriophage. The screening is repeated several more times, each time using the bacteriophage isolated from the previous cycle of screening. Outline the steps you would take to characterise the diversity present in the final "tight binding" population of bacteriophage. Based on the diversity observed, comment on how you might identify the tightest binding bacteriophage in the population. [20%]

(TURN OVER

3 Assume you are a metabolic engineer working on the pathway shown in Figure 1 (in which reactions (arrows) between intermediate metabolites named A-F are catalysed by enzymes named E_x).

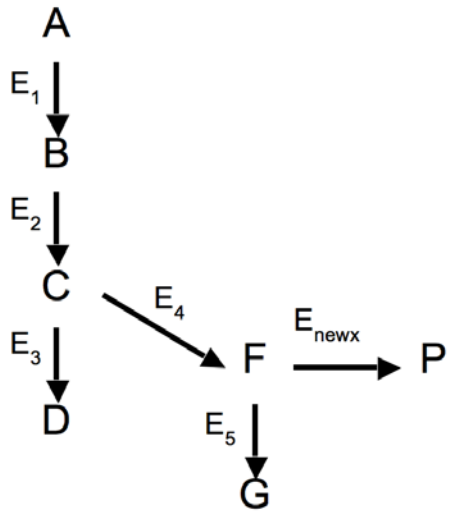

Figure 1.

You are trying to maximize production of the product, P. Enzymes E_1 through E_5 are part of the endogenous pathway native to your host organism, but the organism is not capable of producing P without the introduction of one of several possible foreign enzymes, E_{newx}. You have a collection of DNA parts at your disposal (Table 1) including E_1-E_5, three versions of E_{newx} from different organisms with different Michaelis constants (Km, Table 2) but the same V_{max}, and a collection of promoters including three constitutive promoters with different expression levels (P_{high}, P_{med}, P_{low}) and a repressible promoter (P_{rep}) that can be repressed by addition of the chemical REP. You also have access to the transcription factor TF1 that will up-regulate the expression of E_1-E_5.

(a) You plan to introduce E_{newx} by transforming your host organism with a plasmid built from your collection of parts. Draw that plasmid and explain why you chose the parts that you did. [30%]

(b) Explain how you would go about designing a set of plasmids to maximise the production of P. You can make and experiment with as many plasmids as you like, but in your final system you must use the E_{newx} plasmid designed in (a) plus two additional plasmids, each carrying one gene. [50%]

(Cont.

(c) The host organism is capable of homologous recombination meaning you can replace genomic DNA with parts from your collection. What change(s) might you make to the genome to further maximize production of P? [20%]

Part	Function
E_1	Protein coding
E_2	Protein coding
E_3	Protein coding
E_4	Protein coding
E_5	Protein coding
E_{new1}	Protein coding
E_{new2}	Protein coding
E_{new3}	Protein coding
TF1	Protein coding
P_{high}	Promoter
P_{med}	Promoter
P_{low}	Promoter
P_{rep}	Promoter
Ribosome binding site (RBS)	Translation initiation
Terminator	Transcription termination

Table 1: parts

Enzyme	Km (for substrate F, mM)
E_5	20
E_{new1}	60
E_{new2}	30
E_{new3}	10

Table 2: Michaelis constants

(TURN OVER

4 Combinations of inducible repressors and activators can be used to control transcription and build biological logic gates. Table 3 is a registry of biological parts. Note that all promoter parts have ribosome binding sites (RBS) associated with them. In your answers to the following questions you should choose parts from Table 3.

(a) (i) Draw the designs for two different AND gates and the corresponding truth tables. You should minimise the use of the same parts in each of the two designs. [40%]

(ii) Describe briefly how each of the designs from (i) work. [30%]

(b) Modify one of the designs from (a) to make a NAND gate, give the corresponding truth table and explain the modification. [30%]

(cont.

Table 3: parts to be used in Question 4 design exercises.

Part	Function	Description
pArsR	Arsenic promoter	Sequence covers RNA polymerase binding site.
pLac	*Lac* promoter	Two LacI binding sites on either side of RNA polymerase binding site, allowing a loop to form preventing RNA polymerase to bind in the absence of IPTG.
pAra	Arabinose promoter	In the absence of arabinose, inhibits transcription by blocking RNA polymerase. In the presence of arabinose, promotes transcription by stabilising RNA polymerase binding.
pLac/Ara	Hybrid *Lac*/*Ara* promoter	The two adjacent upstream sites for AraC remain and the downstream site is eliminated so AraC can only act as an activator. The two sites are in between two LacI binding sites so that RNA polymerase can only bind when AraC is bound and LacI is not bound.
pC1	constitutive promoter	Binds RNA polymerase with high efficiency.
pC2	constitutive promoter	Binds RNA polymerase with high efficiency.
pT7	T7 polymerase promoter	Highly specific for T7 RNA polymerase binding and does not bind cellular RNA polymerase.

(TURN OVER FOR CONTINUATION OF TABLE 3

Part	Function	Description
LacI	Lac repressor	Protein tetramer forms DNA loop to prevent RNA polymerase binding. When IPTG is bound to the repressor protein, the loop is opened, allowing RNA polymerase to bind.
AraC	Arabinose repressor/activator	Protein dimer forms DNA loop to prevent RNA polymerase binding. When arabinose is bound to repressor protein, the dimer changes conformation and stabilises RNA polymerase to behave as a transcriptional activator.
ArsR	Arsenic repressor	Protein dimer is specific for pArsR and covers RNA polymerase binding site, preventing RNA polymerase binding. When arsenic is bound the repressor is released from the DNA allowing RNA polymerase to bind.
T7 polymerase DNA binding fragment	This protein fragment binds T7 promoter	In conjunction with *T7 polymerase RNA polymerase activity fragment*, will form a complete protein and transcribe from the T7 promoter.
T7 polymerase RNA polymerase activity fragment	This protein fragment has RNA polymerase activity	In conjunction with *T7 polymerase DNA binding fragment*, will form a complete protein and transcribe from the T7 promoter.
GFP	fluorescent reporter protein	The protein fluoresces green under UV light

END OF PAPER