Version SB/3

EGT2
ENGINEERING TRIPOS PART IIA

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Monday 4 May 2026 9:30 to 11:10

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**Module 3G1**

**MOLECULAR BIOENGINEERING I**

*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 cover sheet.*

**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 at the start of the exam.

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

## You may not remove any stationery from the Examination Room.

1 An *Escherichia coli* strain has been engineered to constitutively express bicyclobutanase, a novel engineered haem-derived enzyme. Bicyclobutanase has a function that is new to nature: it can generate bicyclobutanes, strained ring molecules which are important in medicinal chemistry. This reaction usually require powerful chemical catalysts and replacement with biocatalysis is a step towards more environmentally-friendly production methods but due to the nature of the reaction and the need for bulk synthesis, large amounts of the enzyme will be required per reaction.

The enzyme is being expressed from a high copy number plasmid and during initial characterisation, the strain produces high levels of bicyclobutanase (5mg/L) and grows at an acceptable rate.

(a) After approximately 50 generations of continuous culture, the productivity of the strain declines substantially, despite no change to growth conditions.

(i) Explain why a loss of productivity over time might be expected given the relationship between genotype, phenotype, and selection pressure. [10%]

(ii) List the main components of a protein expression plasmid and briefly describe their role in maintaining expression of the enzyme. [15%]

(iii) Outline an experimental strategy to determine whether the loss of productivity is primarily due to reduced transcription, reduced translation, or loss/inactivation of the enzyme itself. Your answer should reference the appropriate molecular biology techniques and the outcome you would expect in each case. [15%]

(b) You are asked to redesign the plasmid system to improve long-term stability. Propose two changes you could make that might reduce the rate at which loss-of-function variants emerge or take over. For each change, discuss one trade-off or limitation it introduces. [20%]

(c) If it is not possible to design expression systems that produce sufficient bicyclobutanase to be economically viable as a catalyst, another approach is to improve the catalytic activity or the stability of the enzyme. This reduces the amount of enzyme needed to obtain the same yield of product. Often this involves changes to the active site pocket or catalytic residues to improve activity, or to the enzyme surface and the inter-domain interactions to improve stability.

(i) Name and briefly define the four levels of protein structure and the type of bond which stabilises them. [10%]

(ii) Explain the experimental approach you would take to generate and screen new enzyme variants. [5%]

(iii) Describe the main experimental steps in your approach and the appropriate controls to include. [15%]

(iv) Briefly describe two technological reasons that successful engineering of enzymes with new-to-nature functions is more common than in previous decades. [10%]

2 A transgenic strain of *Drosophila suzukii*, a major agricultural pest, has been developed for environmental release as part of a population-control strategy. The strain carries a transgene designed to reduce the viability of offspring when crossed with wild-type flies. This should, over time, reduce population size and crop damage in areas where releases take place. The transgene can be deactivated in the lab because the promoter is repressed by tetracyline added to the fly media, but it is active in the environment in the absence of antibiotic.

(a) After release, it is necessary to monitor the environmental spread of the transgene. PCR can be used on DNA extracted from *Drosophila suzukii* captured in the environment. You design primers that generate a 1546 bp product from transgenic flies and a 236 bp product from wild-type flies.

(i) Briefly explain the principles of PCR and why it is a suitable molecular assay for this application. [10%]

(ii) Using a diagram, describe the base-pairing and interactions of the target DNA and primers at each step in a PCR cycle. [10%]

(iii) After gel electrophoresis, you observe bands at both 1546 bp and 236 bp in single *Drosophila suzukii* individuals sampled from the environment, while a lab-validated transgenic positive control produces a single band. Give two explanations for this result. [10%]

(b) Some genetic systems, such as gene drives, increase the spread of a transgene by ensuring that more than 50% of offspring inherit the gene (i.e. more than expected under standard Mendelian inheritance). This reduces the number of insects required for a successful release and thus the operations costs of the control programme.

(i) Describe one example of a gene drive mechanism. [5%]

(ii) Describe three biosecurity or public-perception objections that different stakeholders might raise regarding the release of insects carrying a gene drive system. [15%]

(c) During the release phase, it is useful for transgenic flies to have survival advantages that increase their likelihood of successfully mating compared to wild-type flies. *Drosophila suzukii* can only tolerate relatively low levels of dietary ethanol. As it breeds on rotting fruit, increasing this tolerance might confer a survival advantage. You consider two ways to increase ethanol tolerance: i) directed evolution; ii) overexpression of recombinant alcohol dehydrogenase, which is an enzyme that metabolises ethanol.

(i) Briefly explain the concept of directed evolution. [5%]

(ii) Outline a directed-evolution strategy to increase ethanol tolerance in Drosophila suzukii. [15%]

(iii) How would you track the genetic changes introduced during the directed evolution campaign? [5%]

(iv) You decide to try the overexpression of alcohol dehydrogenase. This involves generating a new promoter-enzyme fusion in a plasmid that enables CRISPR-based integration into the *Drosophila suzukii* genome. Assuming that you are using a Type IIS Assembly-compatible plasmid and you *de novo* synthesised the DNA components with the appropriate overhangs and restriction sites, describe the key steps in the protocol to generate a sequence-validated plasmid. [15%]

(v) Explain why directed evolution is likely to be more effective than rational design for improving ethanol tolerance in this system. [10%]

3 A well-studied synthetic genetic circuit is the repressilator, a ring of transcriptional repressors arranged in a negative-feedback loop that generates oscillations in gene expression. You are given a bacterial chassis strain growing exponentially in a defined medium with a doubling time of 30 minutes. A 3-protein repressilator implemented in this chassis produces precise oscillations with a period of 480 minutes. A modified design of this oscillator uses a 5-protein repressilator, increasing circuit complexity and burden. Oscillations are monitored using a fluorescent protein reporter with finite maturation and dilution kinetics.

(a) Explain qualitatively how the expression of a synthetic gene circuit can affect the doubling time and volume increase rate of the carrier cell, and interpret the observed period for the 3-protein repressilator. [10%]

(b) You can use time-resolved microscopy to quantify the rate change of volumes of individual cells over time as they grow and divide.

(i) Describe the expected pattern of cell volume (or length) change versus time when a single lineage of the chassis strain is imaged at 5-minute time resolution over several generations. [10%]

(ii) Explain how the presence and operation of a repressilator circuit within these cells could modify these single-cell growth trajectories. [10%]

(iii) Explain why using a much slower imaging interval (e.g. one image every 20 minutes) would be problematic for interpreting both growth dynamics and oscillator behaviour in these cells. [5%]

(c) Fluorescence time traces from single cells carrying the repressilator are measured.

(i) Do you expect the oscillations to be symmetric and sinusoidal, like a harmonic oscillator? Why or why not? [10%]

(ii) The fluorescent protein reporter has finite maturation time and is diluted by growth. Explain how these properties modify the observed fluorescence signal relative to the underlying protein dynamics of the oscillator. [10%]

(iii) For the 5-protein repressilator, what is the expected inter-peak interval in any particular colour channel (assume that the expression of each fluorescence reporter is being regulated by individual repressors from the repressilator circuit)? [10%]

(iv) How do you expect the inter-peak interval estiamte to change, if we add a degradation tag to the reporter to speed up its removal rate? [5%]

(d) Cells carrying the 5-protein repressilator are plated sparsely on agar to form colonies derived from single cells.

(i) Describe the spatial or temporal fluorescence patterns you might observe as the colony expands. [10%]

(ii) Would you expect cells within a single colony to remain phase-synchronized or gradually desynchronize? Why? [10%]

(iii) You acquire time-lapse images of colonies from cells plated from the liquid culture at regular intervals. How can we estimate the doubling time of cells in the culture from colony images? [10%]

4 Some temperate bacteriophages (e.g., Lamba phage) can follow two alternative developmental pathways upon infecting a bacterial cell. In the lytic state, the phage expresses genes that replicate its genome and lyse the host. In the lysogenic state, the phage integrates into the host bacteria's chromosome and expresses a repressor that keeps the lytic genes off while the host continues to grow. Entry into lysogeny is controlled by a genetic toggle switch consisting of two mutually repressing proteins (e.g., CI and Cro in Lamba phage). This circuit produces bistability, allowing the phage genome to stably maintain one of the two states.

(a) The phage toggle switch consists of two transcriptional repressors that inhibit each other. Explain why temperate phages use two repressors rather than two activators for the switch. [10%]

(b) Explain (with a sketch) why a single activator with a high Hill coefficient (large n) cannot reliably replace the two-repressor toggle switch. [10%]

(c) Consider a simplified deterministic model of mutual repression:

(i) Write the expressions for the model for each repressor. [10%]

(ii) Qualitatively sketch or describe the nullclines (where one variable's rate of change is zero, giving you plots of each repressor's abundance as a function of the other repressor) when n = 1 and discuss whether we expect bistable states to appear. [10%]

(iii) Describe (with a qualitative sketch) how the nullclines change when n is increased (e.g., n more than 3) and if we expect bistability to appear. [10%]

(d) During lysogeny, the lysogenic repressor must be constitutively expressed, imposing a metabolic burden on the cell. Assume the lysogen produces 20 molecules per min of repressor protein, and the dilution/degradation rate is 0.02 per min.

(i) Estimate the steady-state copy number of the lysogenic repressor. [10%]

(ii) If the cell contains 100,000 total protein molecules, estimate the percentage of proteome occupied by the repressor. [5%]

(iii) Discuss how this proportion of proteome allocation could affect the growth rate of lysogens and their competitive fitness in mixed populations containing uninfected cells. [10%]

(e) Imagine you are engineering a next-generation therapeutic phage virus whose infection decision is controlled by a synthetic toggle switch. The phage must: a) remain dormant (lysogenic) in healthy carrier cell and b) switch to lytic growth only under infection-associated signals.

(i) Propose necessary modifications to the canonical two-repressor toggle switch described above to achieve this behaviour. [10%]

(ii) If the infection associated signalling molecule cannot pass through the membrane of the chassis cell, what design changes are necessary? [10%]

(iii) How would you acquire the necessary parts for this new design? [5%]

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