23CGP086

Fundamentals of Biotechnology and Genetic Engineering

Semester 1 2023/24 In-Person Exam paper

This examination is to take place in-person at a central University venue under exam conditions. The standard length of time for this paper is **2 hours**.

You will not be able to leave the exam hall for the first 30 or final 15 minutes of your exam. Your invigilator will collect your exam paper when you have finished.

Help during the exam

Invigilators are not able to answer queries about the content of your exam paper. Instead, please make a note of your query in your answer script to be considered during the marking process.

If you feel unwell, please raise your hand so that an invigilator can assist you.

You may use a calculator for this exam. It must comply with the University's Calculator Policy for In-Person exams, in particular that it must not be able to transmit or receive information (e.g. mobile devices and smart watches are **not** allowed).

Attempt **THREE** questions in total. Each question carries 25 marks.

Candidates should show full working for all calculations and derivations.

1. (a) Provide two examples of energy rich compounds that are widely used in cellular metabolism. [2 marks]

(b) A bacterium can use the following redox couples as shown in Table Q1 (b). Evaluate which combination of redox couples will generate the highest energy yield. [6 marks]

Table Q1 (b): Reduction potential of three redox couples.

Redox couple	Reduction potential, E_0' (V)	No of electrons transferred during
CO_2/ glucose	-0.43	24
Pyruvate/ lactate	-0.19	2
Fumarate/ succinate	+0.03	2

The standard free energy change of a redox reaction can be estimated using the Nernst equation:

$$\Delta G_0' = -nF\Delta E_0'$$

where the symbols have their usual meanings and $F = 96.5\ kJ.V^{-1}$.

(c) Provide three important differences between DNA and RNA. [3 marks]

(d) Explain the 'wobble' concept. Examine its role during the coding of the same amino acid by different codons. [6 marks]

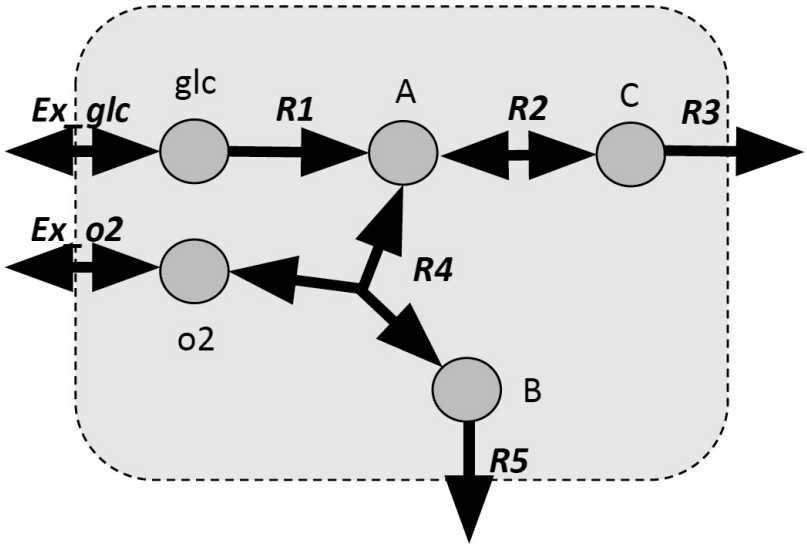
(e) Describe with the help of a schematic diagram the steps of elongation cycle of translation or protein synthesis. [8 marks]

2. (a) Define a metabolic pathway and metabolic pathway flux. [4 marks]

(b) Explain how the pathway flux through a metabolic pathway can be estimated. [2 marks]

(c) Define a stoichiometric matrix and identify its various components. [3 marks]

(d) Consider the metabolic reaction network, as shown in Figure Q2 (d), of a hypothetical cell that uses glucose (glc) and oxygen (o2) to produce a product C. Ex_glc, Ex_o2, R2, and R4 are reversible reactions while the rest are irreversible reactions. The metabolic fluxes through the reactions are represented by v_{glc}, v_{o2}, v_1, v_2, v_3, v_4, and v_5, respectively.



Network Reactions

Ex_glc: glc <=(v_{glc})=> 0

Ex_o2: o2 <=(v_{o2})=> 0

R1: glc ->(v_1) A

R2: A <=(v_2)=> C

R3: C ->(v_3) 0

R4: A + o2 <=(v_4)=> B

R5: B ->(v_5) 0

Figure Q2 (d): Metabolic reaction network of a hypothetical cell.

(i) Write down the mass balance equations for all metabolites, i.e., glc, o2, A, B, and C. [5 marks]

(ii) Determine the metabolite concentration vector, the stoichiometric matrix with upper and lower limits for each reaction, and the metabolic flux vector for the metabolic reaction network. [5 marks]

(iii) If the uptake fluxes for glucose (v_{glc}) and oxygen (v_{o2}) are 1 mmol.h^{-1} and 6 mmol.h^{-1}, respectively, determine the product formation flux (v_3) under aerobic and anaerobic conditions. [6 marks]

3. You are part of a genetically engineered vaccine research and development team working on a new vaccine to prevent Norovirus infection in elderly care home settings. Norovirus is more commonly called the winter vomiting bug and it is highly contagious, resulting in severe vomiting and diarrhoea illness in elderly patients and frequent lock down of care homes to all visitors. Development of a vaccine aims to provide a level of protection against the most severe infections in the most vulnerable patients and against transmission within the care home environment. You are provided with a sample of Norovirus nucleic acid to develop the vaccine from. The Norovirus genome consists of single stranded RNA approximately 7.5 to 7.7 kilobases (kb) in length.

(a) How would you determine whether the sample is pure and acceptable for use as starting template nucleic acid for amplification? [4 marks]

(b) For vaccine development, justify your selections for:

(i) The type of amplification reaction. [2 marks]

(ii) The type of cloning vector for delivery of genes to the host cell. [2 marks]

(iii) The type of host cell for production. [2 marks]

(c) Figure Q3 (c) shows the results from analysis by gel electrophoresis of three different polymerase chain reactions to amplify the Norovirus genome. The PCR reaction mix from the reaction shown in lane 3 and reaction conditions are shown below:

Reaction Mix: Total volume of 50 µL containing 29.6 µL of sterile nuclease-free water, 5.0 µL of PCR enhance mix, 5.0 µL of 10× PCR Buffer (final pH 7.0), 5.0 µL of deoxynucleotide triphosphate mix (4 mM final concentration of each dNTP), 1.0 µL $MgCl_2$ (0.5 mM final concentration), 1.0 µL of genomic template RNA, 100 pmole or 1.0 µL of each oligonucleotide primer (T_M 50°C), and 0.4 µL (1 unit, U) of Taq DNA polymerase.

Thermocycling Conditions: 30 cycles of denaturation at 95°C for 45 seconds, 50°C annealing for 45 seconds and 72°C extension for 1 minute. Final extension at 72°C was performed for 1 minute (1 cycle) and samples were then held at 4°C until required for use (<1 hour).

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Q3 Continued/…

(i) Analyse the success of the results shown in Figure Q3 (c). [5 marks]

(ii) Analyse the PCR reaction mix and reaction conditions and explain what parameters could have led to the different results shown in lane 2. [10 marks]

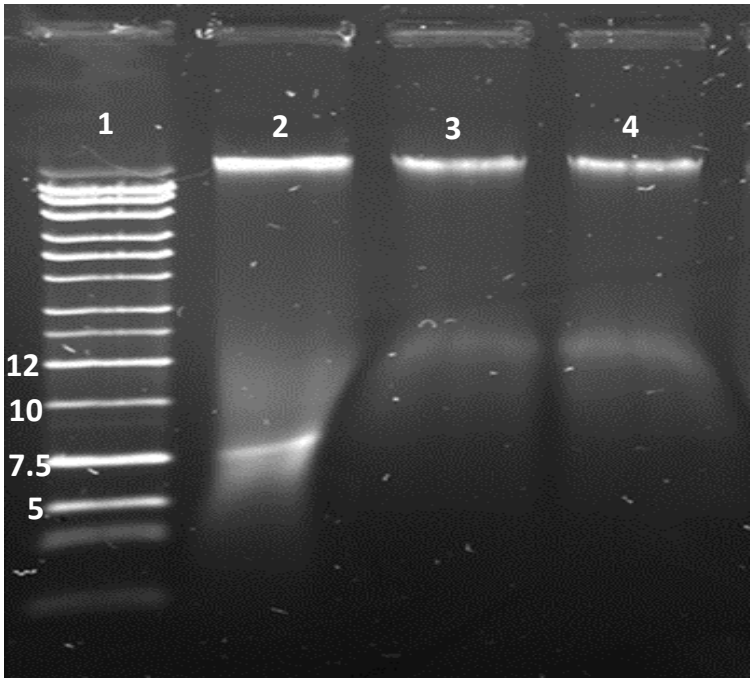

Figure Q3 (c): Agarose gel electrophoresis of polymerase chain reactions (lanes 2- 4) alongside a molecular weight ladder in kilobases (lane 1).

4. (a) Explain the concept of ‘design space’ in pharmaceutical quality by design (QbD) and discuss its significance in pharmaceutical manufacturing. Provide an example of how design space is determined and utilised in the manufacture of a specific drug product. [6 marks]

(b) Examine the role of process analytical technology (PAT) in the pharmaceutical QbD approach. Provide example of a PAT tool and discuss how this tool contributes to real-time quality monitoring and process optimisation in pharmaceutical manufacturing. [5 marks]

(c) You are leading a team of scientists in a reputed biopharmaceutical company, where your team has been tasked to develop a new drug product. As an experienced bioengineer with years of experience in drug development, describe the typical steps that you would recommend your team to follow for the systematic development of the new drug using the QbD principles. Provide an overview of each step. [8 marks]

(d) Table Q4 (d) is showing the historical calibration values of different pH probe performances deemed to be stable and of good quality. You are then asked to set specification limits, so that you can rule out the faulty pH probes before introducing them into your processes. Note that the average values of pH slope and offset are 2014 and 31904, respectively, while the standard deviations for slope and offset are 131.7 and 708, respectively.

(i) What will be the upper and lower limit of specifications for the pH slope and offset? [2 marks]

(ii) Explain if any of the pH probes in Table Q4 (d) would fail your proposed specifications. [2 marks]

(iii) Given a pH probe with a slope of 1617 and an offset of 31500, explain if this would be classified as a good quality probe or not. [2 marks]

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Q4 Continued/…

Table Q4 (d): pH probe calibration values.

Experiment Date	Slope	Offset
04-Feb-18	1971	32038
18-Feb-18	2143	32657
28-Feb-18	1948	32021
09-Apr-18	2053.6	31990
06-Jun-19	2123	32261
11-Jun-19	2123	32261
09-Jul-19	1854.7	31021
30-Aug-19	1716.3	30447
04-Sep-10	1963.3	31038
23-Mar-20	2107	32407
16-Aug-20	2018.7	31938
15-Sep-20	2146.3	32770

END OF PAPER

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