24CGC824
Biochemical Engineering

Semester 1 2024/25 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).

Sections A and B are **COMPULSORY** – attempt **ALL** parts of Question 1. Attempt **BOTH** questions from Section B.

Part A carries 25 marks. Part B carries 50 marks.

Candidates should show full working for calculations and derivations.

Section A

This question is compulsory

1. You are part of a genetically engineered vaccine development team working on a new vector 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) For vaccine development, justify your selections for:

   (i) the type of amplification reaction. [3 marks]

   (ii) the type of cloning vector for delivery of genes to the host cell. [3 marks]

   (iii) the type of host cell for production. [3 marks]

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Q1 Continued/…

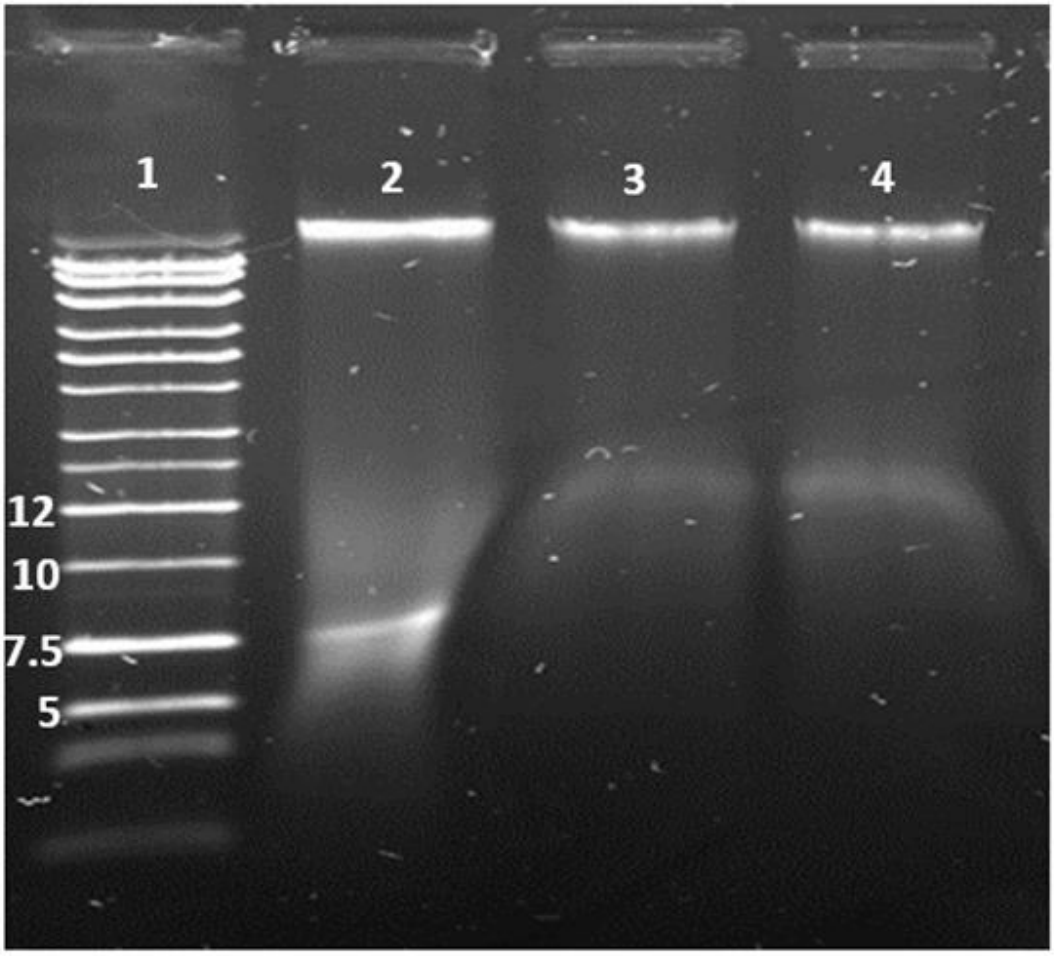

Figure 1. Agarose Gel Electrophoresis of polymerase chain reactions (lanes 2-4) alongside molecular weight ladder in kilobases (lane 1).

Figure 1 shows the results from analysis by gel electrophoresis of three different polymerase chain reactions to amplify the Norovirus genome.

(b) Analyse the success of the results and state which reaction you would take forward for optimisation and why. [6 marks]

Continued/…

Q1 Continued/…

The PCR reaction mix from the reaction shown in Lane 3 (Figure. 1) 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, 1 µL of each oligonucleotide primer (TM 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).

(c) Analyse the PCR reaction mix and reaction conditions and explain what optimisation of parameters could improve the results shown in Figure 1. For each parameter you select, briefly explain why there is a potential issue with the parameter and how it can be improved (1-2 sentences). [10 marks]

Section B

These two questions are compulsory

2. Monoclonal antibodies (mAbs) can be utilised for the treatment of many diseases including cancer. A mammalian cell line has thus been selected for the production of a specific type of mAb (Bevacizumab) for tumour treatment.

   (a) Based on the discussions about cell culture using bioreactors in the lectures, further analyse the issues that should be considered when a bioreactor is designed for the culture of this mammalian cell line. [7 marks]

   (b) If the mammalian cell line is anchorage-dependent, and a hollow fibre bioreactor has been selected for small scale cell culture, recommend a suitable bioprocess to seed and then culture the cells using this hollow fibre bioreactor. [7 marks]

   (c) If the mammalian cell line is anchorage-dependent, and a stirred tank reactor (STR) has been selected for the production of Bevacizumab, (i) in addition to a suitable culture medium, what else is needed for the cell culture? (ii) in addition, propose a suitable bioprocess to seed and culture the cells in the selected bioreactor. [11 marks]

3. (a) Evaluate the advantages associated with solid-state fermentation (SSF) in comparison with traditional submerged fermentation or liquid state fermentation. [11 marks]

   (b) As over 400 species of fungi can attack and kill nematodes and insect pests, solid state fermentation (SSF) has been used for the production of fungal spores for insect control. A packed-bed reactor is selected to produce the fungal spores at pilot-scale. Explain the main components of this bioreactor system and advise how it can be used for the culture of fungi. [7 marks]

   (c) (i) Briefly compare and contrast the first-, second- and third-generation biofuels. [3 marks]

   (ii) With the aid of a schematic diagram, propose a general process for the production of third generation biofuels. [4 marks]

END OF PAPER

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