24CGP086

Fundamentals of Biotechnology and Genetic 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).

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

Candidates should show full working for calculations and derivations.

1. (a) Define biotechnology. Provide two examples of historical and current applications of biotechnology. [5 marks]

(b) Compare and contrast genes and chromosomes and describe their roles in the cell. [5 marks]

(c) Name the three types of RNA involved in protein synthesis and describe their functions. [6 marks]

(d) Consider the following sequence of DNA:

3’ – GGCACCATGCGTCGAAAATCAAAGTGAAACACGGG – 5’

(i) Transcribe the DNA sequence to generate the mRNA sequence. [2 marks]

(ii) Mention the number of codons included in the mRNA sequence and determine the number of amino acids that can be coded by the codons. [2 marks]

(iii) Locate and mention if there is any ‘start’ or ‘stop’ codon present in the mRNA sequence by using Table Q1 (d). [2 marks]

(iv) Using Table Q1 (d), determine the amino acid sequence encoded by the mRNA sequence. [3 marks]

Table Q1 (d). Three letter codons of genetic codes

First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU, UUC: Phe; UUA, UUG: Leu	UCU, UCC, UCA, UCG: Ser	UAU, UAC: Tyr; **UAA Stop**; **UAG Stop**	UGU, UGC: Cys; **UGA Stop**; UGG Trp	U C A G
C	CUU, CUC, CUA, CUG: Leu	CCU, CCC, CCA, CCG: Pro	CAU, CAC: His; CAA, CAG: Gln	CGU, CGC, CGA, CGG: Arg	U C A G
A	AUU, AUC, AUA: Ile; AUG Met	ACU, ACC, ACA, ACG: Thr	AAU, AAC: Asn; AAA, AAG: Lys	AGU, AGC: Ser; AGA, AGG: Arg	U C A G
G	GUU, GUC, GUA, GUG: Val	GCU, GCC, GCA, GCG: Ala	GAU, GAC: Asp; GAA, GAG: Glu	GGU, GGC, GGA, GGG: Gly	U C A G

2. (a) Mention the name of two biological databases from where sequence data for ‘Genomic DNA’, ‘mRNA’, and ‘Protein’ can be obtained. [3 marks]

(b) Define the ‘Reference Sequence’ (RefSeq) database. Explain the differences between ‘model RefSeq’ and ‘known RefSeq’ records with examples. [5 marks]

(c) Explain the ‘Needleman and Wunsch’ algorithm of the dynamic programming method of pairwise sequence alignment. Mention the steps involved in the algorithm. [5 marks]

(d) Construct the dynamic programming matrix, F by using the scoring matrix, S and a gap penalty of 8 to find the global optimal pairwise sequence alignment between the two sequences ‘HEA’ and ‘PAW’, as shown in Figure Q2.1 (d) and Figure Q2.2 (d). [9 marks]

	Gap	**H**	**E**	**A**
Gap				
P				
A				
W				

Figure Q2.1 (d). The dynamic programming matrix, F.

	H	**E**	**A**	**G**	**A**	**W**	**G**	**H**	**E**	**E**
P	-2	-1	-1	-2	-1	-4	-2	-2	-1	-1
A	-2	-1	5	0	5	-3	0	-2	-1	-1
W	-3	-3	-3	-3	-3	15	-3	-3	-3	-3
H	10	0	-2	-2	-2	-3	-2	10	0	0
E	0	6	-1	-3	-1	-3	-3	0	6	6
A	-2	-1	5	0	5	-3	0	-2	-1	-1
E	0	6	-1	-3	-1	-3	-3	0	6	6

Figure Q2.2 (d). The Scoring matrix, S.

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You can use the following relationship to obtain the values of F:

$$F(i,j) = max\begin{Bmatrix} F(i-1,j-1) + S(i,j) \\ F(i,j-1) - d \\ F(i-1,j) - d \end{Bmatrix}$$

where i and j represent columns and rows, respectively of F and S, and d is the gap penalty.

(e) Using the constructed dynamic programming matrix, F, find the optimal global pairwise sequence alignment between the sequences ‘HEA and ‘PAW’, and calculate the optimal alignment score. [3 marks]

3. Despite the fact that gene therapy is still considered a relatively new industry, viral vector platforms have emerged as a preferred platform for gene delivery. Several viral vector-based gene therapies are currently in various stages of pre-clinical testing and clinical trials or have, in fact, already received marketing approval. Challenges include standardisation of processes, optimisation of downstream processing systems, and most notably the scaling of processes to reach the increasing demand of the industry. Viral vector generation typically requires the use of production cell lines and transient transfection, where the vector is passively taken up by the cells over several hours-to-days by providing it in the medium the cells are growing in. Transfection is a unit of manufacture where improvements in standardisation and efficiency are needed for scalability. This is especially true with regards to lentiviral vector production in human embryonic kidney cell lines, where therapeutic development for the treatment of numerous diseases and disorders, from cystic fibrosis, immune disorders, Parkinson's, Alzheimer's to recent COVID-19 research, are in progress.

   You are a senior scientist working on the optimisation of transfection efficiency for lentiviral production. Figure Q3 shows the results from an experiment that looked at two different transfection protocols and four different detection primers. Samples of transfected cells were checked for the presence of integrated lentivirus by Polymerase Chain Reaction (PCR) using lentivirus specific primers and the isolated mRNA from the cells. Four different primer sequences were investigated as probes to amplify a section of lentiviral nucleic acid 200 base pairs (bp) in length. The PCR amplified products were analysed by agarose gel electrophoresis.

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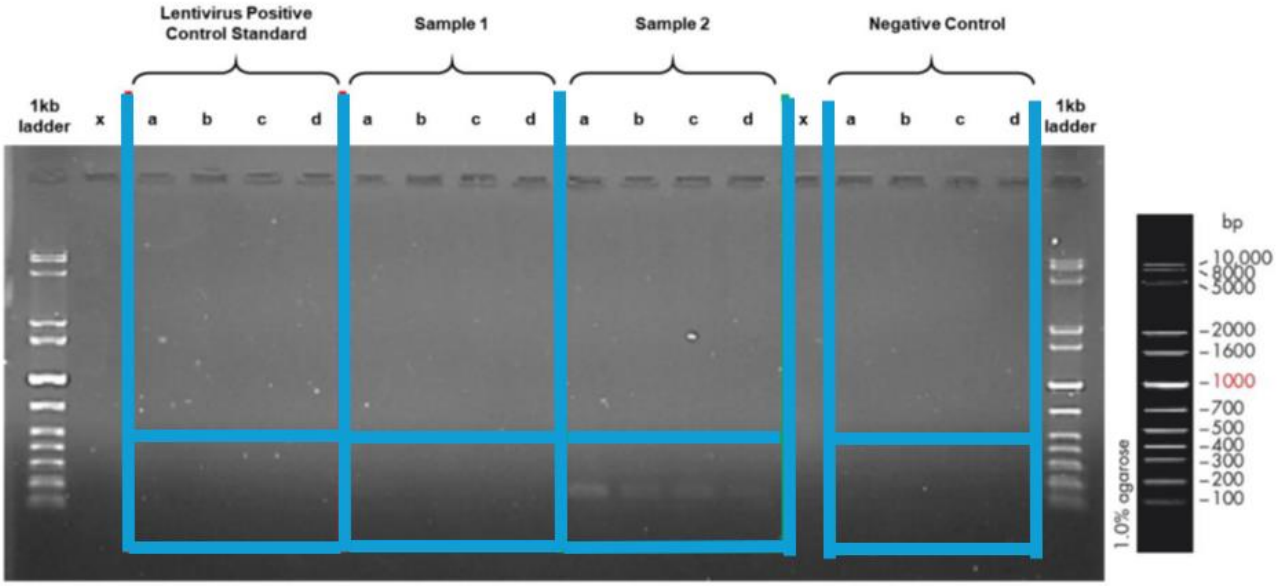

Figure Q3. 1% agarose gel electrophoresis of PCR amplified products from two different transfection protocols (sample 1, sample 2) alongside positive and negative controls. The GelPilot 1 kb Plus ladder was run at each end of the gel with bands identified by molecular weight on the right side of the image. X indicates empty wells. A-D indicate four different primer sequences investigated as probes to amplify presence of lentivirus. Blue columns separate each sample and control, blue boxes indicate where bands of interest may or may not be present.

(a) Which sample and primers would you take forward for further optimisation and why? [4 marks]

(b) Analyse the success of the agarose gel electrophoresis results in Figure Q3 and provide reasoning for improvements you would make. [10 marks]

(c) Explain why a specific type of polymerase chain reaction (PCR) and related primers are needed for detection of lentivirus presence from the cell samples. [5 marks]

(d) Analyse where the critical points are for control within the PCR. [6 marks]

4. (a) Define a genome-scale metabolic network reconstruction (GENRE) and mention its applications in biotechnology. [6 marks]

(b) A biopharmaceutical company is developing a new monoclonal antibody (mAb) for a rare autoimmune disease from a cell culture process. During the early development phase, the company's bioprocess engineering team's preliminary characterisation studies have identified 'glycosylation' as a potential Critical Quality Attribute (pCQA).

Explain how the team can implement the pharmaceutical Quality by Design (QbD) principles to ensure 'glycosylation' patterns are within acceptable limits. [5 marks]

(c) A supplier has informed the team that there will be a change in the raw materials used for cell culture media in the mAb manufacturing process. Preliminary testing shows a slight impact on the cell growth rate and product titre due to this change in the raw materials.

(i) Explain the steps by which the team would assess the impact of this raw material supply variability on the process and product using the pharmaceutical QbD principles. [5 marks]

(ii) Suggest the measures by which the team can adjust the process design or controls to accommodate this change without compromising the product quality. [3 marks]

(d) You are a scientist in a bioprocess development group. A clinical material supply batch run failed, and the root cause was identified to be a faulty pH probe. Consequently, you have been tasked with setting up specification limits to rule out faulty pH probes from future batch runs. Table Q4 (d) shows historical calibration values of different pH probe performances deemed to be stable and of good quality with a pH Slope and Offset average values of 2014 and 31904, respectively. In addition, the standard deviation values for the Slope and Offset are 131.7 and 708, respectively.

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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

(i) Determine the upper and lower limit specifications for the pH Slope and Offset. [2 marks]

(ii) Evaluate if any of the pH probes shown in Table Q4 (d) would fail the specifications determined in (i). Justify your answer. [2 marks]

(iii) Assess and explain if a pH probe with a Slope of 1617 and an Offset of 31500 can be classified as a good quality probe. [2 marks]

END OF PAPER

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