UNIVERSITY OF BOLTON

SCHOOL OF CLINICAL AND BIOMEDICAL SCIENCES

BSC (HONS) MEDICAL BIOLOGY

SEMESTER ONE EXAMINATION 2021/2022

MOLECULAR GENETICS

MODULE NO: BIO5008

Date: Wednesday 12th January 2022 **Time: 10am – 1pm**

INSTRUCTIONS TO CANDIDATES:

Candidates are advised that the examiners attach importance to legibility of writing and clarity of expression. YOU ARE STRONGLY ADVISED TO PLAN YOUR ANSWERS

This examination paper carries a total of 100 marks.

This examination is THREE hours long.

There are TWO sections on this paper.

Section A: Answer ALL questions.

Section B: Answer TWO questions.

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Answer **ALL** questions in Section A and **TWO** questions from Section B.

Make use of labelled diagrams where appropriate.

Section A – answer ALL questions

A cloning experiment has taken place in which a gene of interest has been inserted into a plasmid vector in preparation for transformation into *E.coli* cells. Once successfully transformed, the plasmid DNA was miniprepped from the *E. coli* cells and prepared for sequencing. The plasmid vector was sequenced at its Multiple Cloning Site (MCS) to ensure that the gene of interest had been inserted into the vector successfully. The sequence returned was as follows (Figure 1):

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  1- GAATTCTGTC GGTTGGCGCA AAACACGCTG ATTTTTTCAT CGCTCAAGGC
 51- GGGCAAGCTT ATGGGTGGTA TCAGTATTTG GCAGTTATTG ATTATTGCCG
101- TCATCGTTGT ACTGCTTTTT GGCACCAAAA AGCTCGGCTC CATCGGTTCC
151- GATCTTGGTG CGTCGATCAA AGGCTTTAAA AAAGCAATGA GCGATGATGA
201- ACCAAAGCAG GATAAAACCA GTCAGGATGC TGATTTTACT GCGAAAACTA
251- TCGCCGATAA GCAGGCGGAT ACGAATCAGG AACAGGCTAA AACAGAAGAC
301- GCGAAGCGCC ACGATAAAGA GCAGGTGTAA GTCGACTGAC CGGCTTTGTT
351- TAATCATCAT
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Figure 1: DNA sequence

1. Describe the steps involved in the process of bacterial transformation with a plasmid vector. Specifically, explain the importance of making *E. coli* cells competent and why it is necessary to include a “Heat shock” step.

5 marks

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The gene of interest (Figure 1) was cloned into the plasmid vector using the following restriction enzymes and these have been underlined in the annotated DNA sequence in Figure 1:

HindIII- 5′...A▼AGCTT...3′ / 3′...TTCGA▲A...5′

SalI- 5′...G▼TCGAC...3′ / 3′...CAGCT▲G...5′

2. Using Figure 1, calculate the size of the gene of interest using the two restriction sites provided. Include the restriction sites in the total fragment size.

3 marks

3. Using the restriction sites as a guide, design the appropriate forward and reverse primers needed to amplify the gene in Figure 1 via PCR. The primers should contain the HindIII and SalI sites respectively. Calculate the TM (TM= 2(T + A) + 4(G + C)) and the GC content for each primer.

10 marks

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4. The gene of interest from Figure 1 was originally amplified from genomic DNA using a Polymerase Chain Reaction (PCR).

a) In your answer book, state what A to E represent for a standard PCR protocol on the thermocycler:

	Steps	Temp. for amplification (°C)	Purpose of step
1	Initial denaturation	B.	Initial denaturation breaks H-bonds and separates doubles stranded DNA into single stranded DNA.
2	Cyclic denaturation	95	Cyclic denaturation ensures single stranded DNA remains for every cycle.
3	A.	55	D.
4	Cyclic extension	C.	E.
5	Final extension	72	Allows for the completion of any partial copies and the clearance of all replication machinery from the nascent DNA
6	Storage	4	Prevents degradation by enzymes and high temperatures.

Steps 2 to 4 were repeated 35 times

7 marks

b) Explain both the role of DNA primers in PCR and why they are required.

5 marks

Total 30 marks

Please turn the page

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Section B – answer TWO questions

5. Discuss in depth the term “gene editing tool” and give two examples of such techniques, explaining how they could each be used to cure a well-known genetic disorder of your choice.

35 marks

6. Summarise the structure of DNA and outline the **THREE** experiments that first pointed to DNA being the genetic material in cells.

35 marks

7. Outline our current understanding of the flow of genetic information. Specifically, discuss the processes of transcription, translation and reverse transcription.

35 marks

8. Mutations in the sequence of DNA of a gene can often lead to mistakes in the protein that is expressed. Explain how these mutations can alter the genetic code and give examples of genetic diseases caused by non-functioning protein variants.

35 marks

Total 70 marks

END OF QUESTIONS

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