(SS06)

UNIVERSITY OF BOLTON

SCHOOL OF CLINICAL AND BIOMEDICAL SCIENCES

BSC (HONS) MEDICAL BIOLOGY

SEMESTER ONE EXAMINATION 2022/23

MOLECULAR GENETICS

MODULE NO: BIO5008

Date: Monday 9th January 2023 **Time: 10 – 12.30**

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 75 marks.

This examination is TWO hours and 30 MINUTES 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

Andy has set up an experiment in which he wishes to clone the insulin gene, into a pUC plasmid vector. He starts by extracting genomic DNA (gDNA) from rat cells and uses it as his template for PCR to amplify the gene of interest. Into each primer, Andy incorporates two restriction sites, upstream and downstream of the gene, that correspond to the restriction enzymes HindIII and SalI. Both the PCR product and the plasmid vector are then cut with both enzymes to prepare them for cloning.

1. The restriction enzymes HindIII and SalI both produce “sticky ends” in the PCR product and plasmid vector. Explain the difference between “sticky ends” and “blunt ends”, and why “sticky ends” are advantageous in molecular cloning?

5 marks

2. Andy also wants to clone the insulin gene into the pBad18 protein expression vector using HindIII and SalI. Before he does so, he needs to carry out a restriction mapping exercise to see if it is possible. Answer the following questions on restriction mapping:

   a) A digest of pBad18 is carried out using the enzyme HindIII. The uncut vector is 7.0 kB in size. Cutting the plasmid with HindIII produces 2 fragments. When run on an agarose gel these produce bands of 5.0 kB and 2.0 kB respectively. Draw the plasmid map with the restriction sites and fragments correctly annotated.

2 marks

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b) Using the same plasmid, and digesting with SalI produces also 2 fragments. When run on an agarose gel these produce bands of 6.0 kB and 1.0 kB respectively. Draw the plasmid map with the restriction sites and fragments correctly annotated.

2 marks

c) As Andy requires the plasmid to be cut with both enzymes he preforms a double digest. This results in an agarose gel showing bands of 3.0kB, 2.0kB, 1.0kB. Draw the plasmid map with the restriction sites and fragments correctly annotated, and comment on whether you think it would be suitable to use following the double digest of the plasmid.

6 marks

[Total 10 marks]

3. Following the restriction digest with SalI and HindIII, Andy carries out a ligation reaction to insert the gene fragment into the pUC plasmid vector. He then uses the reaction to transform *E. coli* competent cells. Describe in detail the steps involved in the process of bacterial transformation and why they are necessary. Explain why only a successful ligation reaction will result in a successful transformation of *E. coli* cells.

10 marks

[Section A: Total 25 marks]

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Section B – answer TWO questions

1. Discuss in depth the different levels of structure found in proteins. With that in mind, give two detailed examples of how mutations in the genetic code disrupt protein structure and can give rise to genetic disease.

25 marks

2. Alzheimer's disease is often thought of as an age-related disorder (ARD). Describe how Alzheimer's leads to neurodegeneration and outline how our growing understanding of genetics suggests that genes may contribute more than we thought to ARDs.

25 marks

3. Over the years, the advancement of molecular genetics has been due to the development of many of the components of the "molecular toolkit". In as much detail as possible describe the function of the following and how they are used in molecular genetics:
    - DNA ligase
    - Reverse transcriptase
    - CRISPR/Cas9
    - *Drosophila melanogaster*
    - DNA polymerase

[5 marks each. Total 25 marks]

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4. The use of genomics in medicine is a novel concept in healthcare. Using examples to support your answer, discuss how it is currently used and ways it may be used in the future to improve medicine.

25 marks

5. Explain how bacterial cells regulate their gene expression, using the Lac and Trp operons as examples to support your answer.

25 marks

[Total 50 marks]

END OF QUESTIONS