Instructions

Section A is mandatory. You must answer all of Question 1 in section A.

In Section B, please answer any 2 Questions. Calculators are permitted.

2 hour exam

SECTION A

(You must answer all parts in question 1; worth 40 marks)

Question 1

(a) Discuss the importance of including controls in a Polymerase Chain Reaction (PCR).

(b) Name a restriction enzyme of your choice, in addition to the sequence it recognises and cleaves.

(c) Calculate how much salt you would need to make up 400mL of 190 mM NaCl (MW NaCl is 58.44).

(d) How many microlitres are there in:

(i) 0.87mL

(ii) 2.7mL

(iii) 1 Litre

(e) Discuss the importance of the TATA box in gene promoters and draw a diagram that illustrates the initiation phase of transcription.

(f) Explain what a 'response element' in a gene is.

(g) When making up an agarose gel, the % agarose is important.

(i) If you were asked to make up 50mL of a 0.7% gel, how much agarose would you require?

(ii) Describe why 0.7% is suitable for certain analyses (while 2% may be suitable for others).

(iii) The SYBR safe stain has been provided to you as a 10,000 X liquid stock. What volume of SYBR safe will you add to your 50mL gel?

(h)

(i) Approximately how many genes exist in the human genome?

(ii) Can one gene encode just one protein? Explain your answer.

(Total 8 x 5 = 40 marks)

SECTION B

(Answer two questions in this section; (2 x 30 = 60 marks))

Question 2

When an mRNA (messenger mRNA) is produced initially in eukaryotes, it is referred to as a precursor mRNA transcript. This precursor mRNA is then processed via the addition of a 5' cap and 3' poly A tail as well as the splicing of introns.

(a) Draw & label diagrams of both a precursor and mature mRNA transcript. (8 marks)

(b) List and explain the three functions of the 5' cap. (5 marks)

(c) Draw a diagram of an intron and indicate the sections and sequences that are conserved. (3 marks)

(d) Provide a detailed overview of the splicing process describing the key players involved and how it occurs. Include diagrams as part of your answer. (14 marks)

(Total: 30 marks)

Question 3

The Polymerase Chain Reaction (PCR) is regularly used in molecular biology to amplify target sequences. In recent times, a version of PCR that allows quantitation has been developed, referred to as real time PCR or quantitative PCR (qPCR). Answer each of the following with regard to qPCR.

(a) In a qPCR reaction, what reaction components determines the specific target region to be amplified? Explain how these components work. (6 marks)

(b) Describe the SYBR green quantification method in qPCR and how this works well during a reaction. (6 marks)

(c) What does the phrase "Cycle Threshold" or "C_t value" refer to? Include a diagram as part of your answer. (6 marks)

(d) What does a low C_t value mean with regard to the target sequence? Explain your answer. (6 marks)

(e) Describe the role of the melt curve in qPCR. How is the melting point determined? (6 marks)

(Total 30 marks)

Question 4

With regard to DNA replication, please **answer all 15 of the following questions** (**each carry equal marks**; *Brief answers/diagrams are all that is required in this question).*

(a) What is the name of the location/region of DNA where replication begins?
(b) Explain the role of helicases.
(c) Explain the role of topoisomerase enzymes.
(d) What is the energy source Helicases require for function?
(e) Explain the critical role of single stranded binding proteins at the DNA replication fork.
(f) Once a replication fork has been established, primase lays down a primer. Please give exact details of the type of enzyme involved at this step and describe this primer in detail.
(g) Draw a diagram of a replication fork where the process of replication can be seen to be taking place.
(h) What enzyme is responsible for extending the primer?
(i) There are two forms of the enzyme in answer (h) involved in replication? Explain the differences between them.
(j) Why is the enzyme named in answer (h) not involved in generating/laying down the primer in replication?
(k) Due to the antiparallel nature of DNA, the process of replication encounters a major problem. Explain this problem (in detail) and also present an overview of how the cell gets around this problem.
(l) Name the enzyme involved in removing the primers laid down at the beginning of replication.
(m) What enzyme is involved in filling the gaps generated when the primer is removed?
(n) Name the enzyme involved in joining the ends of new DNA strands together.
(o) In which stage of the cell cycle does replication take place?

(15 x 2 = **Total 30 marks)**