1.8 The Control of Gene Expression: Test Pack

AQA 7402 · Calculator allowed · about 50 minutes

Total Marks

Name: _____ Date: ____ / ____ / _____

Answer ALL questions. Show all your working.

1 This question tests definitions of core regulatory DNA elements involved in prokaryotic gene control.

(a) State the role of a promoter in transcription. **(1)**

(b) State what an operator is in the context of a prokaryotic operon. **(1)**

(Total for Question 1 is 2 marks)

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2 Oestrogen is a lipid-soluble hormone that can alter the transcription of target genes inside a cell.

(a) Describe how oestrogen brings about the transcription of a target gene, from entering the cell to RNA polymerase acting on the gene. **(4)**

(b) A mutation changes the tertiary structure of the DNA-binding region of this transcription factor. Explain the likely effect on transcription of the target gene. **(3)**

(c) State the term used to describe the region of DNA to which a transcription factor binds to initiate transcription. **(1)**

(Total for Question 2 is 8 marks)

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3 This question concerns mRNA stability and steady state abundance. A gene X produces mRNA that is transcribed at a constant rate R transcripts per minute and degraded with first order kinetics with half-life t1/2. The degradation rate constant k = ln 2 / t1/2.

(a) Derive an expression for the steady state number of mRNA molecules Nss in terms of R and k. **(2)**

(b) If R = 120 transcripts per minute and t1/2 = 5.0 minutes, calculate Nss. Give your answer to 3 significant figures. **(3)**

(c) If a drug reduces the mRNA half-life to 2.0 minutes but leaves R unchanged, calculate the new Nss and state the fold change in steady state mRNA abundance (new/old). Give answers to 3 significant figures. **(3)**

(Total for Question 3 is 8 marks)

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4 This question is based on the core practical technique of the polymerase chain reaction (PCR), which is used to amplify small quantities of DNA in vitro. A forensic scientist, Priya Nandwani, set up a PCR reaction starting with 2.50×10^{-3} ng of target DNA. Assume 100% amplification efficiency, so the amount of DNA doubles at every cycle.

(a) State two components, other than the target DNA, that must be present in a PCR reaction mixture. **(2)**

(b) Show that the mass of DNA present after 20 complete cycles is approximately 2620 ng. **(3)**

(c) Calculate the minimum number of complete cycles needed to produce at least 500 ng of DNA from the same starting mass of 2.50×10^{-3} ng. **(3)**

(d) Suggest one reason why the actual mass of DNA obtained after 20 cycles in a real PCR reaction is likely to be less than the value calculated in part (b). **(1)**

(Total for Question 4 is 9 marks)

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5 A researcher measures binding affinity of a transcription factor TF1 to a promoter fragment using an electrophoretic mobility shift assay (EMSA). They incubate increasing concentrations of TF1 with a fixed amount of labelled DNA probe and measure fraction bound. Data are shown below.

TF1 concentration (nM)	Fraction bound
0	0.02
1	0.12
2	0.20
5	0.43
10	0.67
20	0.85

(a) Estimate the dissociation constant Kd for TF1 from the data by identifying the TF1 concentration at which fraction bound is about 0.5. Give your estimated Kd in nM and justify your choice from the table. **(3)**

(b) Using the model fraction bound = [TF]/(Kd + [TF]) and Kd = 6.5 nM, calculate the theoretical fraction bound at 2 nM and compare with the measured value. Give the calculated fraction bound to 2 decimal places and state the percent error relative to the measured value. **(3)**

(c) Suggest three experimental reasons why the measured fraction bound at low concentrations might be lower than the simple equilibrium model predicts. **(3)**

(Total for Question 5 is 9 marks)

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Mark scheme · 1.8 The Control of Gene Expression: Test Pack

Question 1

(a) promoter is a DNA sequence where RNA polymerase and associated factors bind to initiate transcription oe **B1**

(a) Answer: A DNA sequence where RNA polymerase and factors bind to start transcription.

(b) operator is a DNA site adjacent to a promoter where regulatory proteins such as repressors bind to control transcription oe **B1**

(b) Answer: A DNA site near the promoter where regulatory proteins such as repressors bind to control transcription.

Question 2

(a) oestrogen is lipid-soluble/small enough to diffuse directly through the phospholipid bilayer of the cell membrane oe **B1**

(a) oestrogen binds to a (specific) transcription factor/receptor protein in the cytoplasm, forming an oestrogen-transcription factor complex oe **B1**

(a) the (activated) complex moves into the nucleus (through a nuclear pore) oe **B1**

(a) the complex binds to a specific base sequence in the DNA (a promoter/binding site associated with the target gene), stimulating (or inhibiting) the transcription of that gene by RNA polymerase oe **B1**

(a) Answer: Oestrogen diffuses across the cell membrane, binds to a transcription factor to form a complex, which moves into the nucleus and binds to a specific DNA sequence to stimulate transcription by RNA polymerase.

(b) the mutation changes the primary structure (amino acid sequence), altering the bonds (e.g. hydrogen/ionic/disulfide) that determine tertiary structure, changing the shape of the DNA-binding site oe **M1**

(b) the transcription factor complex can no longer bind (or binds less effectively) to its specific/complementary DNA sequence oe **M1**

(b) transcription of the target gene is not stimulated (or is reduced), so the gene is not expressed / the protein is not produced (or produced at a lower rate) ft **A1**

(b) Answer: The transcription factor can no longer bind to its target DNA sequence, so transcription of the gene is not stimulated and the gene product is not made (or is made at a reduced rate).

(c) promoter (region) oe **B1**

(c) Answer: Promoter

Question 3

(a) set rate of change 0 = R - k Nss or equivalent steady state equation **M1**

(a) Nss = R / k cao **A1**

(a) Answer: Nss = R / k

(b) calculate k = ln 2 / 5.0 = 0.138629... min^{-1} or equivalent **M1**

(b) substitute Nss = 120 / 0.138629... oe **M1**

(b) 866 transcripts (3 s.f.), cao **A1**

(b) Answer: 866 transcripts

(c) calculate new k = ln 2 / 2.0 = 0.346574... min^{-1} **M1**

(c) calculate new Nss = 120 / 0.346574... and fold change = new/old, oe **M1**

(c) new Nss = 346 transcripts and fold change = 0.400 (3 s.f.), cao **A1**

(c) Answer: 346 transcripts; fold change 0.400

Question 4

(a) any two from: (heat-tolerant) DNA polymerase (e.g. Taq polymerase); (two) primers; free (deoxyribonucleotide) nucleotides; a thermocycler to control temperature (max 2 marks) oe **B1**

(a) second correct component from the list above **B1**

(a) Answer: Any two of: heat-tolerant (Taq) DNA polymerase; primers; free nucleotides; a thermocycler.

(b) DNA doubles each cycle, so mass after 20 cycles = starting mass x 2^{20} oe **M1**

(b) 2^{20} = 1 048 576 and 2.50 x 10^{-3} x 1 048 576 shown **M1**

(b) = 2621.44 ng, which rounds to approximately 2620 ng (3 sf) cso **A1**

(b) Answer: Approximately 2620 ng (2621.44 ng before rounding)

(c) correct inequality set up: 2.50 x 10^{-3} x $2^{n} \geq 500$ oe **M1**

(c) rearranged and solved: $2^{n} \geq 200\,000$, so $n \geq \log_2(200\,000) = 17.6$ shown **M1**

(c) minimum of 18 (complete) cycles cao (must be rounded up to a whole number) **A1**

(c) Answer: 18 cycles

(d) any one from: amplification efficiency falls below 100% in later cycles as primers/free nucleotides become depleted; DNA polymerase activity declines over repeated heating cycles; single strands increasingly reanneal with each other rather than with primers, reducing effective amplification oe **B1**

(d) Answer: In practice, reagents (primers/nucleotides) become depleted and enzyme activity declines over many cycles, so amplification efficiency falls below the assumed 100%, giving a lower yield than the theoretical maximum.

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Question 5

(a) recognise that fraction bound 0.5 occurs when [TF] = Kd for this binding equation **M1**

(a) interpolate between values: at 5 nM fract 0.43 and at 10 nM fract 0.67 so Kd between 5 and 10 nM **M1**

(a) Kd approx 6.5 nM, cao **A1**

(a) Answer: Kd = 6.5 nM

(b) substitute fraction = 2/(6.5 + 2) = 2/8.5 = 0.235... **M1**

(b) calculate percent error = (measured - model)/measured x 100 or equivalent and evaluate **M1**

(b) calculated fraction = 0.24; percent error = (0.20 - 0.235)/0.20 x100 = -18% approx, cao **A1**

(b) Answer: Calculated fraction = 0.24; percent error = -18%

(c) loss of activity of TF1 at low concentration due to adsorption to tube walls or partial denaturation **B1**

(c) incomplete equilibration or slow association kinetics so not enough complex formed during incubation **B1**

(c) non-specific binding or probe degradation reducing available labelled probe, lowering apparent fraction bound **B1**

(c) Answer: Possible reasons: loss of active TF1 at low concentration (adsorption/denaturation); incomplete equilibration/slow association kinetics; non-specific binding or probe degradation reducing available labelled probe.

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