1.8 The Control of Gene Expression: 1-hour Pack

AQA 7402 · Calculator allowed · about 60 minutes

Total Marks

Name: _____ Date: ____ / ____ / _____

Answer ALL questions. Show all your working.

1 Small interfering RNA (siRNA) is a short, double-stranded RNA molecule that can be used to control the expression of specific genes.

(a) State what is meant by "gene silencing". **(1)**

(b) Describe how siRNA brings about the silencing of a target gene after transcription. **(4)**

(c) Suggest how siRNA technology could, in principle, be used to treat a cancer caused by overexpression of an oncogene. **(2)**

(Total for Question 1 is 7 marks)

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2 Polymerase chain reaction (PCR) is used to amplify a 1.2 kb fragment. Assume perfect doubling each cycle, but primer-dimer and inefficiencies reduce per-cycle efficiency to 90% effective doubling (i.e. multiply by 1.9 each cycle).

(a) Starting from a single copy of the target, write an expression for the number of copies after n cycles with 90% efficiency per cycle. **(2)**

(b) Calculate the number of copies after 30 cycles. Give your answer in scientific notation to 3 significant figures. **(4)**

(Total for Question 2 is 6 marks)

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3 DNA methylation of a promoter region is measured before and after treatment with a drug. At baseline methylation fraction averaged over the promoter is 0.62 with standard error 0.03 (n = 6). After treatment average methylation fraction is 0.41 with standard error 0.02 (n = 6).

(a) Calculate the percent decrease in mean methylation after treatment (use (baseline - treated)/baseline x 100). Give your answer to 2 significant figures. **(2)**

(b) Using the standard errors given, calculate the standard error of the difference in means assuming independent samples (SEdiff = $\sqrt{SE1^2 + SE2^2}$). Give your answer to 2 significant figures. **(2)**

(c) State whether the change in methylation is likely to be statistically significant at approximately the 95% level by comparing the difference in means to twice the SEdiff. Show your comparison and conclusion. **(2)**

(Total for Question 3 is 6 marks)

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4 A western blot experiment measures protein A expression relative to a housekeeping protein H. Densitometry yields integrated intensity for protein A and H for three biological replicates in control and treated cells as below.

(a) Calculate normalised A/H for each replicate and the mean normalised expression for control. Give mean to 3 significant figures. **(3)**

(b) Calculate mean normalised expression for treated samples to 3 significant figures. **(2)**

(c) Calculate the percent expression in treated relative to control (treated/control x100) to 2 significant figures. **(1)**

(Total for Question 4 is 6 marks)

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5 The table shows the percentage of tumour samples found to contain a mutation in the p53 tumour suppressor gene, for four types of cancer.

Cancer type: percentage of tumours with p53 mutation
Colorectal: 50%
Lung: 65%
Breast: 30%
Ovarian: 80%

(a) Identify the cancer type with the lowest proportion of tumours showing a p53 gene mutation, and suggest one other way, apart from mutation of the p53 gene itself, by which loss of p53 function could occur in these tumours. **(2)**

(b) Explain why a mutation that inactivates p53 is more likely to contribute to cancer if it occurs in a body cell that goes on to divide many times, compared with an identical mutation in a cell that rarely divides. **(2)**

(c) The p53 protein acts as a transcription factor. Explain how a mutation affecting only the DNA-binding domain of p53 (leaving its activation domain unaffected) could still result in complete loss of its tumour-suppressing function. **(2)**

(Total for Question 5 is 6 marks)

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6 The human genome contains approximately 3.2×10^9 base pairs but only about 20 000 protein-coding genes, whereas the human proteome is estimated to contain over 100 000 different proteins.

(a) Protein-coding sequences make up approximately 1.5% of the human genome. Assuming this coding DNA is divided evenly between the 20 000 protein-coding genes, calculate the average number of base pairs of coding DNA per gene. **(3)**

(b) Using the information given, explain how the number of different proteins in the human proteome (over 100 000) can be greater than the number of protein-coding genes (20 000). **(3)**

(Total for Question 6 is 6 marks)

7 Somatic (non-germ-line) gene therapy has been trialled as a treatment for cystic fibrosis, an inherited genetic disorder affecting the lungs. Discuss why gene therapy of this type may not provide a permanent cure for the disorder.

(Total for Question 7 is 6 marks)

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Mark scheme · 1.8 The Control of Gene Expression: 1-hour Pack

Question 1

(a) the prevention/blocking of the expression of a gene, so that no functional protein is produced even though the gene is present in the genome oe **B1**

(a) Answer: Gene silencing is the prevention of expression of a gene, so no (functional) protein is produced even though the gene is present.

(b) double-stranded RNA is cut into short fragments (siRNA) by an enzyme oe **B1**

(b) the two siRNA strands separate; one strand associates with a protein complex (RISC) oe **B1**

(b) the siRNA-protein complex binds to a complementary base sequence on the target mRNA (by complementary base pairing) oe **B1**

(b) this leads to the target mRNA being cut/degraded, so it cannot be translated and no (functional) protein is produced from that gene oe **B1**

(b) Answer: Double-stranded RNA is cleaved into siRNA fragments; one strand binds a protein complex which then binds, by complementary base pairing, to matching mRNA; this causes the mRNA to be degraded so it cannot be translated, silencing the gene.

(c) an siRNA could be designed with a base sequence complementary to the mRNA transcribed from the oncogene oe **M1**

(c) this would lead to degradation of the oncogene mRNA, preventing/reducing translation and therefore production of the (growth-stimulating) protein, reducing the rate of (uncontrolled) cell division oe **A1**

(c) Answer: A complementary siRNA could be designed to target the oncogene's mRNA, causing its degradation so the growth-stimulating protein is not translated, reducing the rate of cell division.

Question 2

(a) recognise per-cycle factor is 1.9 and expression is $N = 1 \times (1.9)^n$ or equivalent **M1**

(a) $N = (1.9)^n$, cao **A1**

(a) Answer: $N = (1.9)^n$ copies

(b) evaluate $(1.9)^{30}$ correctly or using log, oe **M1**

(b) convert to scientific notation correctly **M1**

(b) answer 2.30×10^8 (3 s.f.) **A1**

(b) unitless count or 'copies' accepted, cao **A1**

(b) Answer: 2.30×10^8 copies

Question 3

(a) substitute percent decrease = (0.62 - 0.41)/0.62 x100 **M1**

(a) 34% (2 s.f.), cao **A1**

(a) Answer: 34%

(b) substitute SEdiff = $\sqrt{0.03^2 + 0.02^2}$ **M1**

(b) 0.036 (2 s.f.) **A1**

(b) Answer: 0.036

(c) calculate difference = 0.21 and twice SEdiff = 0.072, oe **M1**

(c) 0.21 >> 0.072 so change is likely statistically significant at about 95% level, cao **A1**

(c) Answer: Difference 0.21 > 2 x SEdiff 0.072, so likely statistically significant at about 95% level.

Question 4

(a) calculate replicate normalisations: 1200/800=1.5; 1350/900=1.5; 1280/850=1.50588... **M1**

(a) calculate mean = (1.5 + 1.5 + 1.505882)/3 **M1**

(a) mean control = 1.50 (3 s.f.), cao **A1**

(a) Answer: Mean normalised control = 1.50

(b) calculate treated normalisations: 760/820=0.92683; 690/810=0.85185; 720/830=0.86747 **M1**

(b) mean treated = 0.882 (3 s.f.), cao **A1**

(b) Answer: Mean normalised treated = 0.882

(c) 59% (2 s.f.), cao **A1**

(c) Answer: 59%

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Question 5

(a) breast (cancer), 30% cao **B1**

(a) epigenetic silencing of p53, e.g. hypermethylation of the p53 gene's promoter, reducing/preventing its transcription (accept: increased degradation of the p53 protein, or mutation in a gene that regulates p53 activity) oe **B1**

(a) Answer: Breast cancer (30%); loss of function could occur through epigenetic silencing, e.g. hypermethylation of the p53 promoter reducing its transcription.

(b) without functional p53, cells with damaged DNA are not repaired and/or are not directed to undergo apoptosis, so further mutations are not eliminated oe **M1**

(b) a cell that divides many times has many more opportunities to accumulate the additional mutations (e.g. in oncogenes) needed for cancer to develop, whereas a cell that rarely divides has few such opportunities oe **A1**

(b) Answer: Loss of p53 means damaged cells are not repaired or removed by apoptosis, so mutations accumulate; a frequently dividing cell has many more opportunities to build up the further mutations needed for cancer than a cell that rarely divides.

(c) if the tertiary structure of the DNA-binding domain is altered, p53 can no longer bind to the specific target DNA sequence(s)/promoters of the genes it normally regulates oe **M1**

(c) transcription of these target genes (e.g. those causing cell cycle arrest or apoptosis) is not activated, regardless of a functional activation domain, so the tumour-suppressing effect is lost oe **A1**

(c) Answer: A changed DNA-binding domain prevents p53 from binding to its target gene promoters at all, so those genes (e.g. controlling cell cycle arrest or apoptosis) cannot be activated, even though the activation domain itself still works, resulting in complete loss of function.

Question 6

(a) total coding DNA = 1.5% x 3.2 x 10^9 = 4.8 x 10^7 (bp) shown **M1**

(a) 4.8 x 10^7 / 20 000 shown **M1**

(a) = 2400 bp (per gene) cao **A1**

(a) Answer: 2400 bp per gene

(b) alternative splicing of pre-mRNA means a single gene's exons can be joined in different combinations, producing more than one mRNA (and therefore more than one polypeptide) from the same gene oe **B1**

(b) post-translational modification (e.g. cleavage of a polypeptide, or addition of chemical groups) of a single polypeptide can produce more than one distinct functional protein oe **B1**

(b) as a result, the total number of distinct proteins in the proteome can be much greater than the number of protein-coding genes in the genome oe **B1**

(b) Answer: Alternative splicing allows one gene to produce several different mRNAs/polypeptides, and post-translational modification can convert a single polypeptide into more than one functional protein, so the proteome contains far more distinct proteins than there are protein-coding genes.

Question 7

Level 3 A detailed, coherent discussion that identifies and explains at least two well-developed reasons why somatic gene therapy is unlikely to be permanent (e.g. finite cell lifespan requiring repeat treatment, and either delivery/immune response difficulties or the restriction to somatic rather than germ-line cells), with clear scientific reasoning throughout. **5-6**

Level 2 The answer identifies at least one valid reason (e.g. treated cells are eventually replaced, or delivery of the vector is difficult) with some explanation, but the discussion lacks detail, or only one reason is meaningfully developed. **3-4**

Level 1 Basic or isolated statements, such as "the treatment might not work" or "cells die", with little scientific explanation of why the effect is not permanent. **1-2**

Level 0 No relevant content. **0**

Indicative content:

- Somatic gene therapy only alters the cells that are directly treated (e.g. lung epithelial cells); it does not affect the patient's egg or sperm cells (germ-line cells), so the effect is not passed on to any offspring and the patient's own reproductive cells still carry the faulty allele.
- Cells lining the lungs are naturally replaced on a regular basis (a limited/finite lifespan); as treated cells die and are replaced by new, untreated cells that still carry the faulty allele, the beneficial effect of the therapy is gradually lost.
- As a result, treatment would need to be repeated at intervals throughout the patient's life to maintain any benefit, rather than providing a single, permanent cure.
- It can be difficult to deliver the vector (e.g. a modified virus or a liposome) to a sufficiently large number of cells throughout the affected tissue (e.g. deep into the lungs) for the treatment to have a significant, lasting clinical effect.
- The patient's immune system may recognise and destroy the vector (particularly a viral vector) after repeated exposure, reducing the effectiveness of, or triggering an immune response to, subsequent treatments.
- There is a risk that the inserted gene integrates into the host genome at a random position, potentially disrupting another functional gene (e.g. a tumour suppressor gene or a proto-oncogene), which is a further safety concern with repeated treatment.
- Germ-line gene therapy, which could in principle produce a permanent, heritable cure, is not currently permitted in humans for ethical and safety reasons, so only the less permanent somatic approach can legally be used.

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