HELICATE · A-LEVEL BIOLOGY · TOPIC BANK

3.8.4 Gene technologies

Mark scheme

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TOTAL MARKS	QUESTIONS
60	6

How to use this mark scheme

;	Separates marking points. Each semicolon marks the end of one creditable point, and each point is worth one mark.
;;	Two marks for this answer (used for calculations).
/	Alternative words or phrases that are credited for the same point.
OR	An alternative answer for the same mark.
( )	Words in brackets are not needed to gain the mark.
max	More marking points are listed than marks available. Award up to the number of marks shown.
Accept	An alternative answer that gains credit.
Reject	A wrong answer. It does not gain credit and cancels a correct point it contradicts.
Ignore	Neither credited nor penalised. Mark the rest of the answer.
ECF	Error carried forward: credit correct working that uses an earlier wrong answer.
AO	Assessment objective: AO1 knowledge and understanding; AO2 applying knowledge; AO3 analysing, interpreting and evaluating.

Where an answer is correct but not listed, credit it if it is biologically accurate and answers the question at the depth the marks require. Right + wrong = wrong: a contradiction cancels a correct point.

Producing DNA fragments and in vivo cloning 3.8.4.1

Question	Marking guidance	Mark	Comments
01.1	Reverse transcriptase;	**1** (1 × AO1)	Reverse transcriptase makes a single strand of DNA that is complementary to the mRNA.

Question	Marking guidance	Mark	Comments
01.2	1. Pituitary cells transcribe/express the growth hormone gene, so they contain many copies of its mRNA (but only two copies of the gene) OR the mRNA is easier to isolate than finding the gene among all the DNA/the whole genome; 2. The mRNA has had its introns removed (by splicing), so the cDNA has no introns, and bacteria cannot remove introns (so they could not make the correct protein from the gene); 3. Restriction endonucleases might cut within the gene OR there may be no recognition sites (for one restriction endonuclease) on each side of the gene;	**2 max** (2 × AO2)	2. **Accept** 'mRNA contains only exons/coding sequences, and bacteria cannot splice' 2. 'No introns' and 'bacteria cannot remove introns' are one reason and gain one mark only **Ignore** 'mRNA is easier to cut' / 'mRNA is shorter' unqualified **Ignore** references to reverse transcriptase being cheaper or faster

Question	Marking guidance	Mark	Comments
01.3	The base sequence of the gene;	**1** (1 × AO1)	A gene machine joins nucleotides in the order given by the base sequence (which can be worked out from the amino acid sequence of the protein). No template DNA, introns or restriction sites are needed.

Question	Marking guidance	Mark	Comments
01.4	1. Promoter allows RNA polymerase (and transcription factors) to bind, so transcription (of the cDNA/gene) can start OR so the gene is expressed (in the bacteria); 2. Terminator causes transcription to stop (at the end of the gene) OR causes RNA polymerase to detach;	**2** (2 × AO1)	1. **Accept** either half of point 1: 'RNA polymerase binds to the promoter' OR 'allows transcription to start' **Ignore** 'switched on/off'; points need transcription, expression or RNA polymerase **Reject** start/stop codons for the promoter/terminator **Reject** references to translation starting/stopping at the promoter/terminator **Reject** DNA polymerase for RNA polymerase

Question	Marking guidance	Mark	Comments
01.5	1. Only bacteria that have taken up a plasmid grow, because the plasmid contains gene R/resistance to antibiotic A OR bacteria without a plasmid are killed by antibiotic A; 2. The cDNA is inserted into gene L (at the recognition site), so gene L cannot code for the (functional) enzyme; 3. So substance X is not converted to the blue product, so white colonies contain the plasmid with the cDNA (recombinant plasmid) OR blue colonies contain plasmids without the cDNA;	**3** (3 × AO2)	1. **Accept** 'transformed bacteria' for bacteria that have taken up a plasmid 2. **Accept** 'gene L is disrupted/inactivated (by the cDNA)' 3. **Accept** 'blue colonies contain plasmids that re-joined without the cDNA' **Ignore** 'the marker gene shows which cells are genetically modified' unqualified

Question	Marking guidance	Mark	Comments
01.6	23(%);	**1** (1 × AO2)	138 ÷ 600 × 100 = 23 **Reject** 29.9% (white ÷ blue)

Question	Marking guidance	Mark	Comments
01.7	1. The same recognition site/base sequence is cut, so the cDNA and the plasmid have complementary sticky ends; 2. (So) DNA ligase can join the cDNA to the plasmid OR ligase forms phosphodiester bonds (between the cDNA and the plasmid);	**2** (2 × AO2)	1. **Accept** 'sticky ends that base pair/match'; 'same ends' alone is not enough 2. **Accept** 'ligase joins the sticky ends' 2. **Ignore** references to hydrogen bonds for ligase **Reject** ligase cuts DNA / makes sticky ends

Question	Marking guidance	Mark	Comments
01.8	1. The genetic code is universal OR the same triplets/codons code for the same amino acids (in bacteria and humans); 2. (The mechanism of) transcription and/or translation is universal/the same in bacteria and humans; 3. The cDNA has no introns, so the bacteria do not need to remove introns/splice the mRNA; 4. The cDNA was made from the growth hormone mRNA, so it has the same codons/base sequence (as the human mRNA);	**2 max** (2 × AO1)	**Accept** 'protein synthesis is universal' for point 2 2. **Accept** 'bacteria have ribosomes (to translate the mRNA)' 3. **Accept** 'the cDNA contains only the coding sequence/exons' **Reject** 'the genetic code is degenerate' as the reason **Reject** 'DNA is universal' unqualified

The polymerase chain reaction 3.8.4.1

Question	Marking guidance	Mark	Comments
02.1	1. Stage 1: high temperature breaks hydrogen bonds (between the two strands), so the DNA strands separate; 2. Stage 2: lower temperature allows primers to bind/anneal (to complementary bases on each strand); 3. Stage 3: (DNA) polymerase joins (DNA) nucleotides (to form complementary strands) OR (DNA) polymerase extends from the primers using (DNA) nucleotides;	**3** (3 × AO1)	A correct relative temperature change with its purpose scores each point; a temperature is not required Any temperature given must be within: 90–95 °C (point 1); 40–65 °C (point 2); 70–75 °C (point 3). A temperature outside its range loses that point 1. **Reject** 'enzyme/helicase separates the strands' 3. Nucleotides must be mentioned. Accept Taq polymerase for DNA polymerase; 'polymerase' alone is enough only if 'DNA polymerase' is written elsewhere in the answer 3. **Reject** RNA polymerase / ligase / reverse transcriptase **Ignore** 'optimum temperature for the enzyme' unless the action of DNA polymerase is described

Question	Marking guidance	Mark	Comments
02.2	Correct answer for 3 marks: 1.29×10^{11};;; Accept for 2 marks: 120×2^{30} OR 128 849 018 880 (correct value not in standard form) Accept for 1 mark: 30 cycles (correct working)	**3** (3 × AO2)	Each cycle = 30 + 30 + 60 + 30 = 150 s; 4500 ÷ 150 = 30 cycles; 120×2^{30} = 128 849 018 880 **Accept** 1.3×10^{11} or 1.288×10^{11} for 3 marks **ECF**: for a wrong number of cycles, n, award 2 marks for 120×2^{n} correctly calculated and given in standard form **Reject** 120 × 2 × 30 for any mark

Question	Marking guidance	Mark	Comments
02.3	1. The two DNA strands have different base sequences OR the strands are complementary (not identical); 2. Each primer has a base sequence complementary to (one end of the section on) one of the strands OR one primer binds to each strand; 3. Primers provide a starting point/sequence for DNA polymerase, so both strands are copied;	**2 max** (2 × AO2)	2. **Accept** 'each primer binds to opposite ends of the section of DNA' 3. **Accept** Taq polymerase for DNA polymerase **Ignore** 'primers stop the strands re-joining' as a reason for two different primers **Reject** 'primers are complementary to each other'

Question	Marking guidance	Mark	Comments
02.4	1. The prediction fits the early cycles: the mass increases rapidly/exponentially (up to about cycle 28), as expected if the DNA doubles each cycle; 2. Mixture A levels off/stops increasing after about cycle 30, so the increase after 40 cycles is much less than 2^{40} times; 3. Mixture B levels off at a lower mass/about half the mass of A, so the levelling off is caused by nucleotides running out/being used up; 4. The starting mass is too small to read from the graph, so the size of the increase cannot be checked directly;	**3 max** (1 × AO2) (2 × AO3)	1. **Accept** any cycle from 26 to 30 for the end of the rapid increase 2. **Accept** any cycle from 30 to 34 for the start of the plateau 3. **Accept** 'primers are used up / DNA polymerase is denatured' for point 3 only if the mixture B evidence is not used 3. **Reject** 'DNA polymerase is used up'; ignore 'DNA runs out' **Ignore** 'there is no increase for the first 18 cycles' as evidence against the prediction: the mass is too small to see on this scale **Ignore** a description of the curves that does not evaluate the prediction

Question	Marking guidance	Mark	Comments
02.5	The transformed cells can also make the protein coded for by the gene;	**1** (1 × AO1)	PCR is faster; in vivo cloning needs restriction endonucleases, ligase and a vector. Transformed cells can transcribe and translate the inserted gene (with a promoter), so the protein can be made as well as copies of the gene.

DNA probes, screening and personalised medicine 3.8.4.2

Question	Marking guidance	Mark	Comments
03.1	1. Probe 2 is a (short) single strand of DNA with a base sequence complementary to (part of) allele **a** (including the base that differs); 2. At the base that differs, the probe is not complementary to allele **A**, so it does not base pair/hybridise (fully) with allele **A**;	**2** (1 × AO1) (1 × AO2)	1. **Reject** 'fragment/short section of DNA' without single-stranded, unless complementary bases are described 1. Allele is required; penalise 'gene' for 'allele' once per answer 1. 'Complementary' alone is not enough; must refer to bases/base sequence 2. **Accept** 'one base mismatch, so fewer hydrogen bonds form and the probe does not stay bound' **Ignore** 'the probe is labelled' (given in the question)

Question	Marking guidance	Mark	Comments
03.2	1. Breaks hydrogen bonds (between the two DNA strands) OR separates the two strands/makes the DNA single-stranded; 2. Exposes the bases, so the probe can bind/base pair/hybridise with the complementary bases (of the allele);	**2** (2 × AO2)	1. **Reject** breaking peptide/phosphodiester bonds 2. 'So the probe can bind' alone is enough for point 2 only if single strands are mentioned in point 1 **Ignore** denaturing the DNA unqualified

Question	Marking guidance	Mark	Comments
03.3	1. Couple Y, because both partners bound both probes, so each has allele **a**/is heterozygous; 2. In couples X and Z, at least one partner bound only probe 1/has only allele **A**, so every child would receive allele **A**;	**2** (2 × AO3)	1. Couple Y with no reason gains no mark 2. **Accept** 'a child must receive allele a from both parents (to have condition T)' for point 2 Allele is required; penalise 'gene' for 'allele' once per answer

Question	Marking guidance	Mark	Comments
03.4	1. Couple Y: each child has a 1 in 4/25% chance of having condition T; 2. Couple Y: the counsellor could explain options such as testing an embryo/fetus with the probes; 3. Couple X: no child will have condition T, but each child has a 1 in 2/50% chance of being a carrier; 4. Children who are carriers could be screened (with the probes) before they have children;	**2 max** (1 × AO2) (1 × AO3)	**Max 1** if only one couple is addressed 1. **Accept** 0.25 or 1 : 3 3. **Accept** 'children could be carriers but would not have the condition' Allele is required; penalise 'gene' for 'allele' once per answer **Ignore** 'tell them not to have children'

Question	Marking guidance	Mark	Comments
04.1	Correct answer for 2 marks: 11.6;; Accept for 1 mark: 25(%) and 2.15(%) OR 13/52 ÷ 9/418 (correct working)	**2** (2 × AO2)	13 ÷ 52 × 100 = 25%; 9 ÷ 418 × 100 = 2.15%; 25 ÷ 2.15 = 11.61 **Accept** answers from 11.3 to 11.7 for 2 marks (eg 11.36 from 2.2%), or 12

Question	Marking guidance	Mark	Comments
04.2	1. (Both alleles code for enzyme E with lower activity, so) drug W is broken down more slowly (in the liver); 2. So the concentration of drug W in the blood stays higher/builds up OR the standard dose is too high for them;	**2** (2 × AO2)	1. **Accept** 'less drug W is broken down' 1. **Accept** 'enzyme E has a different tertiary structure/active site, so fewer enzyme-substrate complexes form' as extra detail **Ignore** 'they have no enzyme E'

Question	Marking guidance	Mark	Comments
04.3	1. For: the risk of serious bleeding rises with the number of **S** alleles (**FF** 2.2%, **FS** 5.9%, **SS** 25%); 2. For: patients with allele **S** (**FS** and **SS**) had 27 of the 36/75% of the serious bleeding cases, so screening identifies most patients at risk; 3. For: patients with allele **S** need a lower dose (3.9 or 2.1 mg compared with 5.4 mg), so the starting dose could be matched to the genotype (personalised medicine); 4. Against: 9 of the 36/25% of serious bleeding cases were in **FF** patients, who have no allele **S**, so screening would not prevent all cases OR other factors (eg other genes, age, diet, other drugs) also affect the risk; 5. Against: 418 of 706/about 59% of patients are **FF**, so for most patients screening would not change the dose, adding cost/time; 6. Against: a probe for allele **S** alone does not distinguish **FS** from **SS**, which need different doses (3.9 and 2.1 mg), so a probe for allele **F** is also needed; 7. Against: only mean doses are given, so the variation in effective dose within each genotype is not known; 8. Against: screening may delay the start of treatment for patients at risk of harmful blood clots;	**5 max** (5 × AO3)	**Max 3** if only points for, or only points against, the suggestion are given **Max 4** unless at least one percentage or proportion calculated from Table 3 is used (eg 25%, 75% or 59%) 2. **Accept** 288 of 706/about 41% of patients would be identified as having allele **S** **Ignore** 'small sample' / 'no statistical test' / 'repeat the study' unqualified **Ignore** ethical points not linked to the data

Genetic fingerprinting 3.8.4.3

Question	Marking guidance	Mark	Comments
05.1	1. DNA (fragments) are negatively charged, so they move towards the positive electrode; 2. Shorter fragments/fragments with fewer repeats/bases move faster/further (through the gel);	**2** (2 × AO1)	1. **Accept** 'phosphate groups are negatively charged'; accept anode for positive electrode 1. **Reject** 'DNA is positively charged' 2. **Accept** 'lighter fragments' / 'fragments with lower mass' 2. **Ignore** 'short' alone without a comparison **Ignore** size / smaller / density

Question	Marking guidance	Mark	Comments
05.2	Male 2;	**1** (1 × AO2)	The two bands in the chick's fingerprint that are not in the mother's fingerprint are both present in Male 2's fingerprint, and in no other male's. Each of the chick's VNTRs comes from one of its two parents.

Question	Marking guidance	Mark	Comments
05.3	1. The same restriction endonuclease cuts at the same recognition sites/base sequences, so differences in fragment length are due only to differences in the VNTRs/numbers of repeats; 2. The same gel means the same conditions (eg voltage/time/gel concentration) for every sample, so the distance moved depends only on fragment length; 3. So the positions of bands in different lanes can be compared (to find matching bands);	**2 max** (2 × AO2)	**Max 1** unless both the same restriction endonuclease (point 1) and the same gel (point 2) are explained 1. **Accept** STR for VNTR 2. **Accept** 'same potential difference/current' for voltage **Ignore** 'to make it a fair test' unqualified

Question	Marking guidance	Mark	Comments
05.4	1. Male Q, because he shares the lowest percentage of bands (22%) with female F; 2. Fewer shared bands means fewer VNTRs in common, so male Q is the least closely related to female F; 3. Offspring are less likely to inherit two copies of the same (harmful recessive) allele OR offspring are more likely to be heterozygous OR less inbreeding; 4. Greater genetic variation, so the population is more likely to survive disease/environmental change; 5. Male P shares 64% of bands, so he is likely to be a close relative of female F;	**4 max** (4 × AO3)	1. Male Q is required for point 1 2. **Accept** STR for VNTR 3. Allele is required; penalise 'gene' for 'allele' once per answer **Ignore** 'Q is fitter/healthier' unqualified

Question	Marking guidance	Mark	Comments
05.5	1. The captive population has less genetic variability/diversity OR fewer different alleles (than the wild population); 2. The captive population may have come from a small number of birds OR has had inbreeding; 3. Bring birds from the wild population (or exchange birds with other captive populations) into the breeding programme; 4. Use fingerprints to choose pairs that share the fewest bands/are least closely related for breeding;	**2 max** (2 × AO3)	**Max 1** from points 1–2 and max 1 from points 3–4 1. **Accept** 'birds in the captive population are more closely related/more genetically similar' 1. **Accept** 'smaller gene pool' **Ignore** 'less variation' unqualified

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Question	Marking guidance	Mark	Comments
06	1. Amplify/copy the DNA (from white blood cells) using PCR (because there is only a small amount of DNA); 2. PCR uses primers, DNA polymerase and (DNA) nucleotides OR heat to separate strands, cool so primers bind, then heat so DNA polymerase joins nucleotides; 3. Cut the DNA with restriction endonucleases (at specific base sequences/recognition sites), leaving the VNTRs intact; 4. Separate the fragments by (gel) electrophoresis, according to length; 5. Add labelled DNA probes that bind/hybridise to the VNTRs; 6. Detect the probes using fluorescence/autoradiography/radioactivity, so the bands can be seen; 7. Compare the band patterns: if all the bands from the blood match the suspect's bands, the blood probably came from the suspect; 8. The number of repeats in VNTRs varies between people, so their fragments have different lengths/move different distances; 9. The probability of two people having the same VNTRs is very low (unless they are identical twins);	**6 max** (6 × AO1)	**Max 4** if no explanation of why fingerprinting can identify a person (points 8 and 9) 1. PCR is required; extracting DNA alone does not gain point 1 2. **Credit** relative temperature changes with their purpose; any temperature given must be within 90–95 °C, 40–65 °C and 70–75 °C. Accept Taq polymerase for DNA polymerase 3. **Accept** 'primers that bind either side of the VNTRs, so PCR copies only the VNTRs' for point 3, but a single description of PCR cannot gain points 1, 2 and 3: the primer alternative gains point 3 only if points 1 and 2 are awarded for other statements 3. **Reject** 'cuts the gene/within the VNTR'; ignore 'cuts out the gene' 4. **Reject** separation by positive charge 6. **Accept** fluorescent primers for point 6. Reject 'X-rays' alone **Accept** STR for VNTR throughout **Ignore** gene machine / reverse transcriptase / plasmids

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