

QUESTIONSHEET 1

reverse transcriptase;
 DNA polymerase;
 vector;
 restriction endonuclease;
 sticky ends;
 DNA ligase;
 recombinant;
 E. coli/any correct example;
 calcium chloride/any appropriate salt;

insulin;
 somatotropin/growth hormone; } either way round

TOTAL 11**QUESTIONSHEET 2**

- (a) (i) suspect 3; 1
- (ii) because the bands match closely to the DNA at the scene of the crime/other individuals' bands don't match; 1
- (b) (i) increase quantity/number of copies of DNA (under investigation)/amplification of DNA; 1
- (ii) used to cut DNA into fragments;
 if use the same restriction endonuclease samples from all suspects are cut at the same/similar places; 2
- (iii) separates cut fragments of DNA;
 according to size; 2
- (iv) probes are DNA strands with complementary sequence to cut fragments;
 labelled using phosphorus-32;
 probes bind to the complementary sequence;
 fragments/radiation detected by autoradiography/using X-ray film;
 specific sequences show up as dark bands; max 3

TOTAL 10

QUESTIONSHEET 3

- (a) any three of:
insulin/somatotropin (accept growth hormone)/alpha-1 antitrypsin/interferon/any other correct examples;;; **3**
- (b) bacteria are very easily cultured in bulk but tissue culture cells usually remain as sheets of cells (which limits their culture size);
bacterial cultures are quick growing but tissue culture is a slow process, (thus bacterial cultures produce the product more quickly);
bacterial cultures usually give higher yields of products (than tissue cultures);
tissue cultures require more complex growth media/are more prone to contamination/infection/more difficult to manage (than bacterial cultures); **max 3**
- (c) a bacterial culture may only survive for a short time/few weeks;
a sheep may survive for several years continually making the product/sheep can make the product for several years; **2**
- TOTAL 8**
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QUESTIONSHEET 4

- (a) (i) messenger RNA is extracted (from human cells);
treated with reverse transcriptase to make copy DNA/cDNA **2**
- (ii) treat human DNA with restriction endonuclease to produce sticky ends;
treat sheep DNA with same restriction endonuclease (to obtain complementary sticky ends);
mix two sets of DNA fragments together;
treat with DNA ligase to seal fragments together; **max 3**
- (iii) DNA construct mixed with sheep cells in tissue culture;
exposed to calcium phosphate/heat shock to make cells take up DNA; **2**
- (iv) neomycin will kill any sheep cells that have not taken in the (recombinant) DNA with the neomycin resistant gene;
remaining cells should contain the alpha-1 antitrypsin gene (and can be cultured in large numbers); **2**
- (b) (i) it could be collected from blood/plasma of the sheep/ it could be obtained from the sheep's milk; **1**
- (ii) by aerosol/inhalation; **1**
- TOTAL 11**
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QUESTIONSHEET 5

- (a) (i) the genes have been isolated and inserted into recombinant DNA;
and multiplied many times to produce identical copies/ref to polymerase chain reaction; **2**
- (ii) DNA is copied from human RNA using reverse transcriptase;
treated with restriction endonuclease to produce DNA fragments with sticky ends;
bacterial plasmid /viral DNA treated with same restriction endonuclease;
to produce DNA fragments with complementary sticky ends;
DNA fragments mixed together and sealed/joined to make recombinant DNA using DNA ligase;
many copies made by polymerase chain reaction/amplification; **max 5**
- (b) (i) viruses/adenoviruses;
plasmid-liposome complexes; **2**
- (ii) using an aerosol/inhaler;
intravenous injection; **2**
- (c) liver;
this is where the gene operates to make alpha-1 antitrypsin; **2**

TOTAL 13

QUESTIONSHEET 6

- (a) extract DNA from *B thuringiensis* and cut with (restriction) endonuclease;
 extract DNA from (suitable) vector and cut with the same (restriction) endonuclease;
 example of vector/virus/*Agrobacterium tumefaciens* (crown gall disease);
 pool two DNA samples and DNA ligase to make recombinant DNA;
 insert rDNA into vector using heat treatment/calcium ions to aid uptake;
 mass culture vector and then infect cotton plants; **max 4**
- (b) Bt gene is present in cotton plant DNA/genome;
 copied onto messenger RNA by transcription;
 attached to ribosomes which enable translation;
 transfer RNA brings specific amino acids to ribosomes;
 reference to use of codons and anticodons to assemble polypeptide;
 final assembly of Bt protein in Golgi body; **max 4**
- (c) gene mutation/point mutation of gene/ref base substitution or equivalent;
 to produce a gene which gives resistance to effects of Bt protein/protects insect gut from Bt protein;
 these insects survive and reproduce, passing on the resistant gene;
 not selected against/less competition since susceptible insects have died;
 thus population can grow at a fast rate/huge population develops, (because crop provides almost unlimited food); **max 4**
- (d) Advantages:
 greater yield/more profit/more food for people;
 better quality food since no insect damage;
 no need to use chemical insecticides/less expense/less pollution;
 Bt insecticide (hopefully) only kills insect pest and no other organisms; **max 2**
- Disadvantages:
 pollen may carry Bt protein and could kill (chewing) insects on contaminated plants;
 reference to disruption of food chains (because of insect links being destroyed);
 pollen may hybridise into other plants/weeds giving them insecticide resistance/selective advantage;
 will probably cause the selection (and flourishing) of resistant populations of the insects it was developed to kill; **max 2**
- (e) it is important for humans to produce enough food to feed the whole human population;
 so if GM crops help to do this in a safe way they should be developed;
 there is a risk that GM crops may cause damage to ecological systems/cause serious risks to the survival of other organisms/
 lead to massive resistant insect/weed population explosions/be harmful to humans;
 thus scientists should proceed with caution/employ stringent testing procedures/not be governed by short term financial gain;
- Reject:
 vague non-scientific statements, such as 'man should not play at being God/man should not interfere with God's creation'. **max 2**

TOTAL 18

QUESTIONSHEET 10

- (a) A: reverse transcriptase;
 B: DNA polymerase;
 C: restriction enzyme/restriction endonuclease; **3**
- (b) the plasmid; **1**
- (c) viral DNA/phage DNA/liposomes/Agrobacterium tumefaciens; **1**
- (d) (i) mix vector and bacteria together;
 treat with calcium chloride solution/give heat shock; **2**
- (ii) culture bacteria on large scale/in industrial fermenter;
 in continuous culture if protein is a primary metabolite/in batch culture if protein is a secondary metabolite; **2**
- TOTAL 9**
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QUESTIONSHEET 11

- (a) (i) extract DNA from potato plant which has shown resistance to (potato leafroll) virus (infection);
 cut DNA into sections with sticky ends using a restriction endonuclease enzyme;
 separate DNA fragments by (gel) electrophoresis;
 treat with alkali to split double helix to single strands;
 blot DNA onto a (nylon) membrane and treat with radioactive gene probe;
 to recognise specific base sequences/thus locating DNA fragments with the required gene;
 locate DNA fragments with X ray film;
 collect by washing from nylon;
 amplify/multiply fragments using the polymerase chain reaction; **max 6**
- (ii) extract DNA from bacterium and separate plasmid DNA;
 by (ultra)centrifugation/(gel) electrophoresis;
 treat with same restriction endonuclease to obtain complementary sticky ends;
 separate fragments by gel electrophoresis;
 use gene probe to identify and discard fragments containing tumour gene;
 use polymerase chain reaction to multiply/amplify remaining plasmid fragments; **max 4**
- (iii) mix plasmid DNA with sticky ends and potato DNA with sticky ends together;
 treat with DNA ligase to seal ends together; **2**
- (iv) mix plasmids in growing culture of the bacterium;
 in presence of calcium ions/apply heat shock; **2**
- (v) culture transformed bacteria and potato tissue/callus together;
 bacteria infect potato tissue and plasmids incorporate into potato cells;
 using plant cell attachment gene;
 callus/culture differentiates into new resistant potato plants;
 can be recognised by effects of marker gene; **max 3**
- (b) insect resistant plants;
 fungal resistant plants;
 pesticide resistant plants;
 improved flavour tomatoes;
 increased shelf life tomatoes; (any correct examples) **max 2**

TOTAL 19

QUESTIONSHEET 12

- (a) gene probe is a length of single stranded DNA used to locate a gene;
 is tagged with a radioactive tracer/fluorescent dye so that it can be located;
 probe contains base sequences complementary to those in the gene/either side of the gene;
 target DNA containing the required gene must be in single stranded state;
 thus when probe and target DNA are placed together they bind (and the gene is marked); **max 4**
- (b) target DNA containing required gene is located using a gene probe;
 short nucleotide sequences either side of the gene are determined and complementary lengths of DNA (oligonucleotides) are chemically synthesised;
 target DNA is heated/heated to 93°C which causes double helix to unwind;
 cooled/cooled to 55°C, oligonucleotides added which bind to complementary sequences (either side of gene);
 DNA polymerase added and temperature raised slightly/to 72°C;
 two new copies of the gene are then made;
 process then repeated, the number of copies doubling each time;
 ref to use of automated process/machine which can produce millions of copies in a few hours; **max 6**
- (c) DNA extracted from blood/semen/any biological material;
 cut into lengths using a restriction endonuclease;
 fragments separated by gel electrophoresis;
 transferred/blotted onto a nylon membrane/ref Southern blotting;
 radioactive DNA probe then applied and attaches to specific base sequences;
 nylon membrane placed in contact with X-ray film to locate radioactive regions;
 banding patterns on different DNA samples can thus be compared for similarity; **max 5**

TOTAL 15**QUESTIONSHEET 13**

Action of enzyme	Named enzyme
Enables transcription of DNA from mRNA	reverse transcriptase;
Enables transcription of mRNA from DNA	RNA polymerase;
Cuts DNA at specific base sequences	restriction endonuclease;
Binds DNA fragments of different origin together	DNA ligase;
Enables polypeptide synthesis from amino acids in the ribosomes	peptide synthetase;
Enables DNA replication in the cell cycle	DNA polymerase;
Used to make multiple copies of DNA in genetic engineering	DNA polymerase;

TOTAL 7

QUESTIONSHEET 14

- (a) (i) DNA fingerprinting; **1**
- (ii) collect chimpanzee mitochondria by cell disruption/(ultra)centrifugation;
extract mitochondrial DNA and split with restriction endonucleases;
separate DNA fragments by gel electrophoresis;
blot onto nylon membrane/Southern blotting;
treat with radioactive/fluorescent gene probe to recognise and label specific base sequences;
overlay with X-ray film to detect labelled sequences; **max 5**
- (iii) labelled areas of DNA show up as dark bands;
if (many) bands do not match those of other subspecies/are different from other subspecies, then they are probably an
independent subspecies; **2**
- (b) forensic science/murder/rape;
paternity disputes;
confirming animal pedigrees;
identification of human remains;
locating genes causing inherited disease;
locating genes for animal/plant breeding; **max 2**

TOTAL 10