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

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

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 7

(a) (i) ——— A A G C T T ——— ;
——— T T C G A A ——— ; **2**

(ii) fragments of DNA formed by a restriction enzyme;
which have dangling/exposed nucleotides
e.g. —TTCGA and AGCTT— ;
which are complementary to each other; **max 3**

(iii) a particular restriction enzyme will cut any type of DNA in exactly the same position;
sticky ends will be complementary so DNA from any organism can be joined to DNA from any other organism; **2**

TOTAL 7

QUESTIONSHEET 8

(a) (i) restriction enzyme/restriction endonuclease; **1**

(ii) enzyme X/same restriction enzyme/same restriction endonuclease; **1**

(b) plasmid/virus; **1**

(c) plasmid/vector and bacterium suspended in (cool) calcium chloride solution;
then heated to 42^0C;
plasmid/vector enters bacterium; **3**

(d) plasmid/vector replicates;
if plasmid/vector contains gene, gene also replicates;
protein coded for by the gene may be synthesised; max **2**

TOTAL 8

QUESTIONSHEET 9

(a) make them toxic to pests/insects/caterpillars/give them a natural insecticide/reduce use of artificial pesticide; **1**

(b) identify sequence of amino acids in toxin;
use genetic code to identify the codons/base sequences;
make complementary radioactive gene probe;
to find those codons in the bacterial DNA; **3**

(c) restriction enzyme/restriction endonuclease; **1**

(d) put genetically engineered plant/leaves from a genetically engineered plant into a container;
set up similar container using ordinary plant/leaves;
expose each to similar number of insects/caterpillar
record leaf damage/consumption/count dead insects (after a few hours); **max 3**

TOTAL 8

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 metabalite/in batch culture if protein is a secondary metabalite; **2**

TOTAL 9

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 <u>same</u> 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 93^0C which causes double helix to unwind;
cooled/cooled to 55^0C, oligonucleotides added which bind to complementary sequences (either side of gene);
DNA polymerase added and temperature raised slightly/to 72^0C;
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