1. (a) Gene is a (length) of DNA;

Gene is a sequence of bases/chain of nucleotides;
Triplet (base) code/read in three"s;
On sense/coding strand;
Triplet coding for amino acid;
Degenerate code; non-overlapping; start/stop code;
Sequence of triplets/bases code for protein; $\max 4$
(b) Restriction enzymes;

Cut DNA; at specific base sequences;
Same (restriction) enzyme also cuts DNA; into which gene is inserted/plasmid/virus/Agrobacterium;
(DNA) ligase;
Joins two pieces of DNA together/forms recombinant DNA;
Vector needed to insert DNA into host/plasmid enters host/second organism;
Correct ref. to sticky ends; Reverse transcriptase; mRNA $\rightarrow$ DNA;
$\max 6$
(c) Unwinding/unzipping of DNA;
involving breaking of hydrogen bonds;
Assembly of mRNA nucleotides;
Complementary base pairing/example;
Role of polymerase enzymes;
mRNA enters ribosomes;
Specific tRNA molecule associated with specific amino acid;
Codon - anticodon relationship;
Formation of peptide bonds;
Specific role of ATP/energy; Reference to gene switched on;
$\max 7$

Quality of language

## Aspect of work

Grammar, punctuation and spelling of an acceptable standard
1
Material presented in an appropriate scientific style with due regard to correct use of technical terms

Argument clearly and logically presented 1
2. (a) Do not produce enzyme G/produce non-functional enzyme G; No linamarin formed;
(b) (i) Reverse transcriptase;
makes single strand of DNA/cDNA from RNA;
Double strand then formed;
DNA polymerase;
$\max 3$
(ii) If from DNA all genes are present in cell; mRNA from activated genes only/codes for one protein;
DNA has introns/non-coding/junk DNA;
Have been edited out in mRNA; $\max 2$
3. (a) (i) Difficulty of finding one gene among all the genes in the nucleus / large amounts of mRNA coding for insulin will be present in insulin producing cells / idea that mRNA will be „edited"
(ii) Reverse transcriptase; 1
(iii) AGTTGG; 1
(b) Joins the gene for insulin into the plasmid; 1
(c) Allows transformed bacteria to be separated from non-transformed; Further detail e.g. transformed bacteria survive when antibiotic applied to medium;
4. (a) Locate with the use of a gene probe;

Use restriction enzymes / endonucleases;
To cut at specific base sequence;
By hydrolysing / breaking sugar-phosphate bonds;
$\max 2$
(b) Same restriction enzymes;

Cut at same base sequence in bacterial DNA;
Leaving sticky ends / hydrogen bonds break;
Join / splice with ligase;
Use of plasmid; $\max 4$
5. Quality of written communication should be considered in crediting points in the marking scheme. In order to gain credit, answers must be expressed logically in clear, scientific terms.
(a) (i) Restriction;
I endonuclease only
(ii) Ligase / ligating;
(b) Cut ends of DNA;
one strand longer than the other / staggered cut / unpaired bases;
Can attach to complementary DNA / bases;
(c) Transfers / carries (foreign) gene / DNA;

Into bacteria / carrot cell / host cell;
(d) Acts as a marker;

Both genes on same plasmid / bacteria take up both genes;
Reference to antibiotic;
Kills bacteria without (either) gene / plasmid /
only those with gene/plasmid will grow / survive;
$\max 3$
(e) For

More effective than other methods;
More humane method than shooting;
Poisons may harm other animals;
Prevent spread of disease / destruction of crops;
Economic benefit to farmer / consumer;
Against
Plasmid / (either) gene may enter another species;
May eradicate / greatly reduce possums;
May sterilise other species;
Disruption of food chain / change in numbers of a species;
Not immediate / only affects next generation;
Reference to antibiotic resistance in bacteria;
Possums resistant to sterility protein; $\max 3$
6. (a) (i) $\mathbf{A}$ - restriction (endonuclease);

B - ligase;
(ii) Cut at same base sequence;

Produce sticky ends;
Idea of complementary bases / complementary shape of sticky ends; max 2
(b) Yeast cells that make insulin have plasmid;
so have respiration gene;
Can respire;
Can reproduce / grow;
A converse points for yeast cells which can't make insulin
7. (a) DNA unwinds / splits / separates / hydrogen bonds break;

To allow assembly of mRNA;
Using mRNA nucleotides;
Via RNA polymerase;
Complementary sequence / or equivalent;
mRNA joins to ribosome;
tRNA carries a specific amino acid;
Codon-anticodon relationship / or explained / defined;
Peptide bonds form between amino acids;
(b) (i) UACCGUA;

## (ii) AUGGCAU

(iii) Needed as a signal for the gene to be transcribed;

Or equivalent - NOT translated
(c) (i) Sequence / bases complementary;

Reference to hydrogen bonds;
NOT 'corresponding' bases
(ii) RNA duplex has uracil / U, DNA has thymine / T;

RNA duplex has ribose, DNA has deoxyribose;
RNA duplex shorter than DNA; 2 max
(iii) Ribose will not fit on double-stranded RNA;

No exposed bases;
Needed for tRNA to attach; 2 max
8. (a) (i) Region of non-coding DNA / degenerate DNA; 1
(ii) $\mathrm{A}-\mathrm{T} / \mathrm{C}-\mathrm{G} \quad 1$
(b) (i) Cut vector / plasmid DNA with restriction enzymes / endonucleases;

Use (DNA) ligase;
To join sticky ends / description;
(ii) (Plasmid) DNA base sequence / gene (function) altered / different proteins made;
(c) (i) Arrow pointing downwards

AND
lightest molecules move the furthest / fastest / ora;
(ii) 5 ;
(iii) Probe binds to complementary base sequence in gene; Position determined by radioactivity / fluorescence;
(d) DNA unzips / unwinds / splits / separates / hydrogen bonds break; To allow assembly of mRNA;
Using RNA nucleotides;
Via RNA polymerase;
Complementary sequence / eq; mRNA joins to ribosome (accept travels to ribosome); tRNA carries a specific amino acid;
Codon-anticodon relationship / explained;
Peptide bonds form between adjacent amino acids; 6 max
9. (a) Different venoms contain different antigens;

Antibodies only fit with antigens of the right shape / complementary / specific;
(b) (i) 3 bases in (RNA / DNA) code for one amino acid;

Work out correct base sequence;
(ii) Some amino acids are coded for by more than one codon / code is degenerate;
Artificial gene may have different codons;
Natural gene may contain introns / junk DNA; 2 max
(iii) 1. DNA separates / hydrogen bonds break; accept unzips NOT unwinds
2. To allow assembly of mRNA;
3. Using (m)RNA nucleotides;
4. Via RNA polymerase;
5. Complementary sequence / or equivalent;
6. mRNA joins to ribosome; allow travels to ribosome
7. tRNA carries a specific amino acid;
8. Codon-anticodon relationship / or explained / defined;
9. Peptide bonds form between amino acids; 6 max
10. (a) Carrier of foreign DNA / gene; 1
(b) (i) Pst I; 1
(ii) (Loss of) marker gene;

Genetic code / base sequence / DNA altered;
(So) gene no longer functional;
(iii) Separate DNA strands to expose sense strand / probe only a single strand;
Probe contains a complementary base sequence to gene;
Attaches to complementary sequence if gene present;
Presence / location indicated by radioactivity / fluorescence;
3 max
(c) So cells cannot conjugate / link;

To stop transfer of DNA;
To reduce risk of other organisms in environment getting altered genes; 2 max

## z t11. Quality of Language

The answer to this question requires continuous prose. Quality of language should be considered in crediting points in the mark scheme. In order to gain credit, answers must be expressed logically in clear scientific terms.
(a) Restriction enzyme:

Any four from:
Cuts (source) DNA / cuts gene;
Cuts plasmid;
By hydrolysis;
Same restriction enzyme used (to cut each type of DNA);
Acts on specific base sequence;
Staggered cut / leaving sticky ends / OR add sticky ends to the cut DNA shown on annotated diagram;
Complementary base pairing between 2 DNA molecules (at sticky ends);

## Ligase:

Splices/joins DNA pieces / forms covalent bonds;
By condensation;
$\max 6$
(b) Any two from:

DNA / plasmid / genes / resistance gene / chromosomes;copied Each daughter cell receives a copy / cells are produced by cloning cells are a clone;
All cells are produced by mitosis;
$\max 2$
(c) (i) Shows which cells/bacteria have taken up the plasmid / which cells/bacteria have taken up the gene; Only modified cells/bacteria / cells/bacteria with the plasmid survive in presence of the antibiotic/are resistant (to antibiotic);
(ii) Not needed here since can select using herbicide in the growth medium herbicide kills non-modified cells/tissue/plant,

Expect better yield of crop plant / reference weeds compete for water/minerals/ ,nutrients"/light; [Allow: 'for $\mathrm{CO}_{2}$ '/ 'for $\mathrm{O}_{2}$ ']
Can kill weeds specifically / in presence of (resistant) crop plant;
Problem:
EITHER: Herbicide/its breakdown products could accumulate up food chains;
Herbicide/its breakdown products may be toxic to other organisms;
OR: Pollen with resistance gene could pass to (related) weed species;
(Could no longer use herbicide as) weeds become resistant; $\max 4$
12. (a) (i) Use of restriction enzyme;
"Cuts" between (specific) bases;
(ii) Plasmid / virus/ microinjection/ tungsten bullets;
(b) Disease caused by toxin;

Only part of toxin made which wonl cause disease;
(c) 1 DNA unwinds/ hydrogen bonds break;

2 to allow assembly of MRNA;
3 Using (m) RNA nucleotides;
4 Via RNA polymerase;
5 Complementary sequence/ or equivalent;
6 MRNA joins to ribosome;
7 TRNA carries a specific amino acid;
8 Codon-anticodon relationship/ or explained/ defined-,
9 Peptide bonds form between amino acids;
$\max 6$
13. (a) Enzyme $1=$ (restriction) endonuclease/restriction (enzyme)/named example; Enzyme 2 = (DNA) ligase ;
(b) Any two from:
(Many) bacteria/cells do not take up the plasmid/gene;
Only bacteria with the plasmid/modified/transformed will survive/grow/multiply;
Since plasmid/bacterium has (gene for) ampicillin resistance;
[Accept: ampicillin tolerance]
(c) (i) C ;
(ii) Gene for growth hormone is inserted in the gene for tetracycline resistance/gene for tetracycline resistance cannot be expressed/unmodified have intact tetracycline resistance gene; Bacteria/cells with hGH gene are killed by tetracycline / unmodified bacteria/cells are not killed by tetracycline;
(d) May confer antibiotic resistance to other/pathogenic bacteria/named example; Can no longer prevent/cure disease/can"t treat patient (with this antibiotic); [Note: Not person made immune]
14. (a) (i) contains genes/nucleotides/sections of DNA/artificial DNA from two species/2 types of organisms;
(ii) carries gene/DNA (into the other organism /gene carrier); 1
(iii) expose cells to the fungus; non-resistant ones die, resistant ones survive; OR identify by adding marker gene/gene probe/(qualified) marker probe; description of positive result e.g. radioactivity/fluorescence / complementary base pairing;
(b) EITHER 1 cut desired gene (from DNA) of oat plant; 2 using restriction endonuclease/restriction enzyme;
OR 1 use mRNA from oat which will code for resistance; 2 and use reverse transcriptase to form desired DNA;
OR $\quad 1$ make artificial DNA with correct sequence of bases; 2 using DNA polymerase; 3 cut plasmid open; 4 with (same) restriction endonuclease/restriction enzyme; 5 ref. sticky ends/unpaired bases attached; 6 use (DNA) ligase to join / ref. ligation; 7 return plasmid to (bacterial) cells; 8 use of $\mathrm{Ca}^{2+} /$ calcium salts/electric shock; $\quad \max 6$ (if ref. to , insulin" allow 5 max.)
15. (a) Presence of resistant and non-resistant varieties / mutation produces resistant variety;
Resistant ones survive / non-resistant ones killed by treatment;
These will reproduce and produce more resistant parasites/pass on resistance allele;
Greater probability of another person being infected by resistant parasites; max 3
(b) Likelihood of being infected (by strain resistant to both drugs) is less; $1 / 500 \times 1 / 500 / 1 / 250000$;
Drug has longer effective life;
(c) (i) As comparison / to show that nothing else in the treatment was responsible;
(ii) Given injections of saline / injection without SPf66; (otherwise) treated the same as experimental group;
(d) (i) $100 \%$; 1
(ii) $10 \%$; 1
(e) (i) Different lengths of DNA have different base sequences / cut at specific sequence;
Results in different shape / different shape of active site; Therefore (specific sequence) will only fit active site of enzyme;
(ii) Recognition sites contain only AT pairs; Which would occur very frequently;
16. (a) (i) Sticky ends/description;

Reference to complementary base-pairing 2
(ii) Ligase; 1
(b) Carrier;

DNA/gene; (context of foreign DNA)
Into cell/other organism/host;
(c) Act as marker gene;

Allows detection of cells containing plasmid/DNA;
Reference to growing bacteria on antibiotic; $\max 2$
17. (a) (i) Sticky ends/description; Reference to complementary base-pairing
(ii) Ligase;
(b) Carrier; DNA/gene; (context of foreign DNA) Into cell/other organism/host;
(c) Act as marker gene;

Allows detection of cells containing plasmid/DNA;
Reference to growing bacteria on antibiotic; $\max 2$
18. (a) (i) Reverse transcriptase; 1
(ii) Idea that mRNA is present in large amounts in cell making the protein / mRNA has been edited / does not contain introns / mRNA codes for single protein;
(b) (Ligase) splices / joins two pieces of DNA / "sticky ends";
19. (a) Endonuclease / restriction enzyme;
(b) DNA made of base pairs;

Each base pair is same length / occupies same distance along backbone;
(c) (i) Second blank box from left labelled 6;
(ii) Distance moved depends on length / number of base pairs / second longest fragment / second shortest distance identified;

1
(d) 5; 1
20. (a) Mother and father both heterozygotes / Tt / carriers; Probability of thalassaemia $1 / 4$ and female $1 / 2$; Probability of both $1 / 8$;
(b) (i) Cut at same base sequence as same enzyme used;

Fragments are same length / size / have same charge;
Only differs by a single base;
(ii) Single base occurs many times;

Sequence of 20 unlikely to occur elsewhere;
Allow one mark for establishing the principle where neither marking point clearly made.
21. (a) 1. DNA is cut;
2. using restriction enzyme;
3. electrophoresis;
4. separates according to length/mass/size;
5. DNA made single-stranded;
6. transfer to membrane/ Southern blotting;
7. apply probe;
8. radioactive/ single stranded/ detected on film/ fluorescent;
9. reference to tandem repeats/VNTRs/minisatellites;
10. pattern unique to every individual;

6 max
(b) cells on toothbrush; DNA present in cell;
(c) (i) toothbrush gives small sample of DNA/ need more DNA for analysis; PCR gives many copies;
(ii) uses heat; to separate strands;
OR
PCR replicates pieces of DNA;
because DNA has been cut;
OR
primer added in PCR;
to initiate replication
(d) (i) PCR/amplification needed; 1
(ii) other DNA present; need to identify ,required" DNA from rest; 2

## 22. General principles for marking the Essay:

Four skill areas will be marked: scientific content, breadth of knowledge, relevance and quality of language. The following descriptors will form a basis for marking.

Scientific content (maximum 16 marks)

| Category | Mark | Descriptor |
| :---: | :---: | :---: |
|  | 16 |  |
| Good | 14 | Most of the material of a high standard reflecting a comprehensive understanding of the principles involved and a knowledge of factual detail fully in keeping with a programme of A-level study. Some material, however, may be a little superficial. Material is accurate and free from fundamental errors but there may be minor errors which detract from the overall accuracy. |
|  | 12 |  |
|  |  |  |
|  | 10 |  |
| Average | 8 | A significant amount of the content is of an appropriate depth, reflecting the depth of treatment expected from a programme of A-level study. Generally accurate with few, if any fundamental errors. Shows a sound understanding of most of the principles involved. |
|  | 6 |  |
|  |  |  |
|  | 4 |  |
| Poor | 2 | Material presented is largely superficial and fails to reflect the depth of treatment expected from a programme of A-level study. If greater depth of knowledge is demonstrated, then there are many fundamental errors. |
|  | 0 |  |

Breadth of Knowledge (maximum 3 marks)

| Mark | Descriptor |
| :--- | :--- |
| 3 | A balanced account making reference to most if not all areas that <br> might realistically be covered on an A-level course of study. |
| 2 | A number of aspects covered but a lack of balance. Some topics <br> essential to an understanding at this level not covered. |
| 1 | Unbalanced account with all or almost all material based on a single <br> aspect |
| 0 | Material entirely irrelevant. |


| Mark | Descriptor |
| :--- | :--- |
| 3 | All material presented is clearly relevant to the title. Allowance <br> should be made for judicious use of introductory material |
| 2 | Material generally selected in support of title but some of the main <br> content of the essay is of only marginal relevance. |
| 1 | Some attempt made to relate material to the title but considerable <br> amounts largely irrelevant. |
| 0 | Material entirely irrelevant or too limited in quantity to judge. |

Quality of language (maximum 3 marks)

| Mark | Descriptor |
| :--- | :--- |
| 3 | Material is logically presented in clear, scientific English. Technical <br> terminology has been used effectively and accurately throughout. |
| 2 | Account is logical and generally presented in clear, scientific <br> English. Technical terminology has been used effectively and is <br> usually accurate. |
| 1 | The essay is generally poorly constructed and often fails to use an <br> appropriate scientific style and terminology to express ideas. |
| 0 | Material entirely irrelevant or too limited in quantity to judge. |

## Additional notes on marking

Care must be taken in using these notes. It is important to appreciate that the only criteria to be used in awarding marks to a particular essay are those corresponding to the appropriate descriptors. Candidates may gain credit for any information providing that it is biologically accurate, relevant and of a depth in keeping with an A-level course of study. Material used in the essay does not have to be taken from the specification, although it is likely that it will be. These notes must therefore be seen merely as guidelines providing an indication of areas of the specification from which suitable factual material might be drawn.

In determining the mark awarded for breadth, content should ideally be drawn from each of the areas specified if maximum credit is to be awarded. Where the content is drawn from two areas, two marks should be awarded and where it is taken only from a single area, one mark should be awarded. However, this should only serve as a guide. This list is not exhaustive and examiners should be prepared to offer credit for the incorporation of relevant material from other areas of study.
23. (a) restriction (enzyme) / endonuclease / named example;
(b) unpaired bases / sticky ends / staggered; complementary / explained;
(c) 1 mark for each correct outcome plasmid with foreign DNA joined in ring; ring with plasmid only; ring of foreign DNA only; ignore linear structures
24. (a) (i) restriction enzyme / endonuclease; cuts DNA at specific / recognition sites / base sequences;
(ii) use of same endonuclease / restriction enzyme;
ligase;
joins DNA strands; vectors e.g. plasmid; $\max 2$
(b) use genetically engineered and normal plants at similar stages of growth; standardise all other factors / named factor e.g. temperature; reference to method used to measure growth e.g. height, mass, leaf area etc.
25. (a) extract DNA;
remove specific section;
using restriction endonuclease
base sequence;
method of finding the base sequence eg gene probe;
compare with normal sequence for gene;
$\max 3$
(b) screening of individuals at risk for presence of markers;
example of individual at risk;
earlier detection of tumours;
earlier surgery/drug treatment;
$\max 3$
26. (a) G and GATCC or CTAG and GATC;

CCTAG
G
1
(b) hydrogen bonds; between bases;
(c) to form same sticky ends/same exposed base sequence; as pairing of bases must occur;
27. (a) use of restriction endonuclease/enzyme; same for human and plasmid DNA;
sticky ends; ligase used to insert/join human to plasmid DNA;
(b) only yeast cells with the enzyme/plasmid/gene can survive;
and only these can produce insulin;
so, any living cells will have the insulin gene;
28. (a) (i) DNA/gene cut from petunia using endonucleases; ligase used to attach gene to plasmid/vector/named vector; detail of suitable method of transfer/reference to cloning gene; (absorption of vectors / microinjection/microfusion/shotgun)
None of points - but idea of cutting and splicing using enzymes: max 1
(ii) active site of enzyme changed so glyphosate unable to enter/ site where glyphosate binds no longer reacts/ reference to a change in the enzyme;
(b) (i) can spray for weeds without damaging crop; may increase yield as less competition from weeds;
(ii) transfer of gene into weeds/
loss of diversity/
toxicity to pollinators/organisms feeding on pollen/plants/ unknown environmental effects;
max. 1
29. (a) use of endonuclease/restriction enzyme;
which cuts DNA either side of gene/at specific point;
(b) fertilised egg divides by mitosis;
which produces identical cells/identical genotype/identical DNA;
(c) only one DNA molecule/chromosome likely to contain new gene/ female heterozygous; half gametes produced contain the gene;
30. positive:
less crops lost to insect damage/ diseases spread by insects;
can spray herbicide with no loss to crop/reduce competition from weeds;
more saleable product;
less use of insecticide;
possibly cheaper food;
negative:
gene transfer to non-crop species;
consumer resistance to "un-natural" products;
transfer of genes into food chains/effect of food chains/examples;
creation of "plague" weeds/uneconomic plants;
excessive use of herbicides;
Reject disadvantages of selective breeding max 3
31. (a) (i) heat (to about $\left.90^{\circ} \mathrm{C}\right) \quad 1$
(ii) primers / short nucleotide chains / RNA added individual (DNA) nucleotides then added; by (DNA) polymerase
(b) 2 double stranded molecules with original (white) and new (black) DNA strands;
2 double stranded molecules with new (black) DNA strands
(c) provides multiple copies of a DNA fragment; e.g. to analyse in forensic detection
32. Max 3 advantages or disadvantages
advantages e.g.:
food stays firm for longer;
allowing shipment;
longer shelf life;
greater profit;
disadvantages e.g.
transfer of mutant gene to bacteria / gut bacteria might become resistant to kanamycin;
more difficult to treat gut infection;
consumer resistance to GM food;
long term effects unknown 5
(c) provides multiple copies of a DNA fragment; e.g. to analyse in forensic detection
33. (a) (i) current switched on / fragments move due to electrical attraction; several hours to run;
DNA transferred to nylon membrane / ,southern blot";
(wrapped) photographic film placed on gel;
film developed / radioactivity darkens film max 4
(ii) different lengths / mass; 1
(iii) e.g. progress towards cure for genetic diseases 1

## (b) Quality of written communication.

The answer to this part of the question requires continuous prose.
To gain one mark for Quality of Written Communication these answers should be presented in clear, scientific English. Technical terminology should have been used effectively and should usually be accurate.
maximum 4 marks for generic genetic engineering techniques:
human gene identified
removed from human DNA using endonuclease;
detail e.g. sticky ends;
same endonuclease;
used to cut plasmid;
role of ligase; $\max 4$
combined with promoter sequence;
gene / DNA (+ promoter sequence) injected into nucleus of fertilised sheep egg;
detail e.g. micropipette;
embryo inserted into sheep uterus;
enzyme obtained from sheep milk. Overall max 6
34. (a) Endonuclease / restriction enzyme

1
(b) To multiple / amplify the fragments

1
(c) Using electric current to separate fragments
(d) Mark this section as a whole
(i) Probes will attach to fragments;

Radioactivity / autoradiography will make fragments visible.
(ii) Probes attach only to fragments with complementary base pairs;

Only these will be radioactive / show up on film.
35. (a) (i) Cells disrupted to remove DNA;

Endonuclease / restriction enzyme cuts DNA;
Reference to specificity;
Reference to sticky ends;
Plasmid cut;
With (same) endonuclease;
Use of ligase;
Treatment of recipient bacteria to make them accept plasmid e.g. heat shock.
$\max 5$
(ii) Fermenter;

Detail;e.g. supplied with appropriate food / oxygen / suitable temperature
(b) (i) Addition / deletion / substitution;

Of nucleotide / base
(ii) mRNA formed;

Detail e.g. RNA polymerase / complementary base pairing / transcription (linked to mRNA formation);
mRNA attaches to ribosomes / rough ER;
tRNA molecules bring amino acids;
Anticodons on tRNA complementary to mRNA codon / translation; Amino acids joined together / peptide bonds.
(iii) Only liver cells have (membrane) proteins /
receptors correct shape for virus to bind onto
1
36. (a) Genetically identical group 1
(b) Use micropipette plasmids / liposomes / virus / tungsten bullets (injection neutral vector / electric shock neutral)
(c) (i) Much faster / easier to obtain since produced by cell division eggs are produced inside sheep in ones or twos /
less risk of virus infection
(allow less risk of rejection)
(ii) To see which cells have taken up gene;

Antibiotic will kill cells where no incorporation
(iii) Pros, e.g.

Embryo cells can develop into any type of tissue;
Easier to use embryo cells than to extract cells from a person;
Cells / DNA would replicate in patient during mitosis;
Permanent cure / one treatment might be sufficient / no need for continued medication;
Likely to be safer than implants from animals;
Since these may contain ,new" viruses;
Only body cells implanted, therefore germ line would not be affected;
Less danger of damage to other genes;

Cons, e.g.
Effects of introduction might not be fully understood / long term effects unknown;Religious / ethical issue explained, e.g. embryo has potential for development to person therefore could be regarded as murder /
,,embryo rights";
(allow reference to antibiotic resistance passed on to microbes)
('we should not play God' neutral)
(reject references to evolutionary effects)
max 4 if only pros or only cons max 6
37. (a) (i) Codon; 1
(ii) Tyrosine; 1
(b) (i) Base sequence / codon (of DNA) is changed;

Different (sequence of bases in) mRNA;
Attracts different tRNA / anticodon;
Different amino acid inserted into protein / polypeptide;
(ii) More than one base triplet / codon codes for one type of amino acid; Suitable example / true for the third base of the codon;
(c) Endonuclease / restriction enzyme cuts DNA;

Reference to specificity sticky ends / use the same restriction enzyme on fragment and plasmid;
Ligase used to fix ends;
(d) Details of taking a replica:
ef filter paper / felt / nylon membrane;
To obtain an exact copy;
Bacteria spread on agar to obtain separate colonies;
Grown on agar containing ampicillin;
Bacteria containing plasmid survive;

For principles and detail of replica plating:
Placed on agar containing tetracycline;
Bacteria growing on ampicillin, but not tetracycline contain
the recombinant plasmids;
Because foreign DNA has been inserted into the tetracycline gene;
38. (a) (i) Production of single / separate stranded DNA;
(ii) Attaches to / complementary to start of the gene / end of fragment; Replication of base sequence from here;
(iii) Enzymes active / not denatured at high temperatures; Allowing rapid replication of DNA;
(b) 256; 1
(c) (i) Large scale / cheap / easier production of human protein; 1
(ii) Long term effects unknown / effects of introducing foreign genes not fully known;
Ethical issue explained - eg wastage of sheep embryos / embryos have potential for development / animal rights, eg wrong to experiment on animals; May encourage similar research using cells from human embyros / that develop into humans;
May spread antibiotic resistance into other species; 3 max
(Playing God / references to evolutionary effect - neutral)
39. (a) replace defective genes/treat genetic diseases with (healthy) genes;
(b) one amino acid missing/different/changed;
(c) (i) gene is expressed;
healthy genes replicated with cells so not lost;
(ii) gamete cells are not affected/do not take up the healthy gene; still able to pass on the defective gene;
40. (a) different recognition sites/base sequences; different active sites;
(b) (i) single stranded/sticky ends/hydrogen bonding; complementary/base pairing occurs;
(ii) different plasmids contain different numbers/sized/types of fragment;
(iii) ligase;
(c) (i) smaller/less dense/lower mass/fragments move further/faster; (not lighter) (allow the converse)
(ii) four bands
identical bottom and middle bands, extra band between these, top band lower;
41. (a) (i) have mechanism for entry into cells/inject DNA into cells/virus can enter cells/virus can infect cells; replicate in cells; target specific cells;
(ii) may cause disease/infection/harm; immunity may develop to the virus/destroyed by immune response or white blood cells;
(b) (i) only has to be treated once/permanent; all cells in body have replaced gene; can be passed to offspring; 1 max
(ii) changes to genetic make-up of individual/species/genome/future generations/germ line;
may affect normal development; (reject - not ethical) 1 max
(c) high cost plus reason e.g. compared with conventional treatments; adverse effects not known; use of animals in (preliminary) testing; other genes introduced which may have damaging effects/damage genome/damage other genes;
42. (short) length of DNA;
single stranded; (reject reference to RNA)
with specific base sequence/complementary base sequence;
indicates where replication starts/stops annealing;
2 max
43. (a) circular DNA;
separate from main bacteria] DNA; contains only a few genes;
(b) enzymes only cut DNA at specific base sequence/recognition site/specific point;
sequence of bases/recognition site/specific point (on which enzyme acts) occurs once in plasmid and many times in human DNA;
(max 1 if no reference to base sequence or recognition site)
(c) all cut DNA have same/complementary base sequence at ends or same/complementary sticky ends; random process by which sticky ends join;
(d) (i) replica plating;
use of pad/velvet surface to transfer bacteria;
use of agar plate containing ampicillin/no tetracycline and agar plate containing tetracycline;
in bacteria with human DNA tetracycline gene no longer functional/ not resistant to tetracycline;
bacteria with human DNA grow on plate with ampicillin/no tetracycline but are killed by tetracycline; bacteria with no extra DNA in plasmid not killed,

4 max
(ii) use of gene probes;
bacteria with insulin gene produce insulin;
1 max
44. (a) gene no longer functional / bacteria not resistant to tetracycline;
(reject gene/plasmid not resistant to tetracycline)
(b) (i) so that bacteria stick to it / transfer of bacteria; 1
(ii) identifies those bacteria with plasmid;
as bacteria without plasmid / ampicillin gene killed;
2
(ii) identifies which bacteria have recombinant DNA/ foreign DNA present / human gene present;
these are killed by the antibiotic;
as the gene for tetracycline resistance has been destroyed / bacteria not resistant to tetracycline;
(c) colony present on ampicillin plate but not on tetracycline plate;
(b) (i) cells/embryos/DNA damaged by process; embryo rejected;1
(ii) gene not incorporated into plasmid/vector; gene/plasmid not incorporated into sheep cells/DNA/chromosomes; gene not switched on/expressed;
(c) (i) meiosis/gamete formation / present in germline cells; fertilisation/fusion of gametes/zygote formation;
(ii) gene in plasmid which is not passed on in the cytoplasm; only one chromosome of pair passed on / gene or allele only on one chromosome;
half the gametes contain the gene; 1 max
46. (a) heat DNA to $95^{\circ} \mathrm{C} / 90^{\circ} \mathrm{C}$;
strands separate;
cool so that primers bind to DNA; add DNA polymerase/nucleotides; use of restriction enzymes; use of electric current and agar/gel; shorter fragments move further;
(b) probes bind to complementary base sequences;
(bands refer to) different base sequences along DNA / same base sequences not repeated along DNA;
47. (a) use restriction enzyme/endonuclease/named, e.g. Bam/Eco; to cut DNA in specific place/base sequence;
(b) heat DNA to $90-95^{\circ} \mathrm{C}$;
strands separate;
add primers;
and nucleotides;
cool so that primers bind to DNA;
(DNA) polymerase forms new strands/joins nucleotides; 4 max
(c) (i) virus is inhaled/sprayed into the lungs; gets into cells, inserting the healthy gene; 2
(ii) makes DNA from RNA, (rather than other way round); 1
48. (i) mRNA attaches to ribosome;
codon on mRNA;
binds to an anti-codon on tRNA;
each tRNA brings a specific amino acid; sequence of codons/bases on mRNA determines order of amino acids; formation of peptide bonds/amino acids joined by condensation reactions;
(iii) inserted gene/mRNA complementary to normal gene/mRNA; binds to it to prevent protein synthesis/form double strand/prevents mRNA binding to ribosomes; will not stop all translation, some mRNA reaches ribosomes/ because not all mRNA is bound by inserted gene mRNA;
49. (a) (i) to separate polynucleotide strands/form single strands;
(ii) not denatured (at $\left.95^{\circ} \mathrm{C}\right)$;
(iii) for binding of primers/nucleotides (to DNA strands);
(b) (i) doubling (of DNA) each cycle;
but very low numbers to start with, so appears flat; then exponential growth;
(ii) suggestion; with explanation e.g.:
nucleotides being used up;
so less/nothing to make complementary chains;
primers used up;
so cannot start complementary chains;
enzymes losing activity/denatured;
so no polymerisation of complementary strands; 2 max
50. (a) (i) number of bases $=4440$ (allow 4446 if they refer to start/stop); each amino acid coded for by triplet/three bases (so three times more bases than amino acids);
(ii) deletion;
(deletion) of three bases;
because substitution/addition would change amino acid(s);
2 max
(b) (i) codon on mRNA;
specific/complementary base pairing with;
anti-codon on tRNA;
specific tRNA for each amino acid;
protein formed by condensation reactions / peptide bonds formed;
(ii) (loss of amino acid) changes tertiary structures/3D shape; so sugar molecules cannot be attached (to form glycoprotein/ functional protein); so (defective) unable to bind to chloride ions/use ATP; 2 max
51. (a) isolate wanted gene/DNA from another organism/mRNA from cell/organism; using restriction endonuclease/restriction enzyme/reverse transcriptase to get DNA;
produce sticky ends;
use ligase to join wanted gene to plasmid;
also include marker gene;
example of marker e.g. antibiotic resistance;
add plasmid to bacteria to grow (colonies);
(replica) plate onto medium where the marker gene is expressed; bacteria/colonies not killed have antibiotic resistance gene and (probably) the wanted gene;
bacteria/colonies expressing the marker gene have the wanted gene as well;

6 max

3 max

1 max
longer but without harmful side effects;
disadvantage e.g.:
takes longer to take effect;
52. (a) introduction of healthy gene / ,replacement ${ }^{\text {tc }}$ of defective gene;
(b) can enter cells / infect cells / inject DNA into cells; targets specific cells; replicates (in cells);
(c) reproductive cells/gamete cells do not contain ADA allele / gene;
(d) (i) to ,prevent" rejection / immune response;
(ii) T lymphocytes have a limited life span / die off / do not reproduce; bone marrow provides continual supply of T lymphocytes / (ADA) gene enzyme;
53. (a) to separate the two strands / break hydrogen bonds;
(b) (i) enables replication/sequencing to start (allow keeps strands separate);
(ii) joins DNA nucleotides (not complementary bases);
(c) (i) 64 ;
(ii) replication of DNA from crime scene/tissue sample / for DNA sequencing / gene cloning;
(d) (transcription uses) RNA polymerase; RNA nucleotides / uracil; one (template) strand / PCR both strands; start / stop codons; 2 max (accept enzyme separates strands)
54. (a) (cut out gene using an) endonuclease / restriction enzyme; reference to specificity / recognition site;
sticky ends;
use the same enzyme to cut;
plasmid / virus / potato DNA;
fixed by ligase;
method of introducing vector e.g. micropipette / virus injects DNA / remove plant cell wall;
(b) introduced gene / characteristic passed to offspring; rapid process; larger number of plants produced; asexual reproduction genetically identical / sexual reproduction causes variation;
(c) different genes are expressed; producing different enzymes/proteins; 2
55. (a) polymerase chain reaction / PCR; 1
(b) (i) joins nucleotide together; (not complementary bases) 1
(ii) enables replication / sequencing to start / keeps strands separate; 1
(c) (i) (modified nucleotide) does not form bonds/react with other nucleotides; 1 does not "fit" DNA polymerase/enzyme/active site;
(ii) AC ;

1 max
(accept reading from right hand side i.e. TC)
(d) (i) different lengths / sizes / mass; 1
(ii) radioactive primer; 1
(iii) GAAGTCTCAG; 1
(accept reading from autoradiogram i.e. CTTCAGAGTC)
56. (a) probe will attach (to mutant allele);
attaches to one DNA strand;
as a result of complementary base pairing;
radioactivity detected on film/X-ray / by autoradiography
(if mutant allele present);
(b) for
gene is only active in mammary cells / only affects milk / easy to obtain product / product produced in large amounts / gene passed to offspring;
against
long term effects not known / qualified reference to animal exploitation e.g. use of embryos / effect of inserted gene on other sheep tissues/genes;
57. (a) (i) restriction (endonuclease) enzyme;
cuts DNA at specific/restriction points/after specific base sequence;
(ii) PCR/polymerase chain reaction; 1
(b) isolated cells divide by mitosis;
can get many plants (producing toxin) / rapid production of (toxin producing) plants;
all cells (in the new plant/clone) will produce the toxin;
only small number of cells in the whole plant would produce the toxin /
express gene;
3 max
58. (a) (i) transfer/carry genes from one organism to another/into bacteria/cells; 1
(ii) cut open plasmid; cut donor DNA, to remove gene/length of DNA; cut donor DNA and plasmid with the same enzyme/enzyme that cuts at the same base sequence; sticky ends/(overhanging) ends with, single strand/bases exposed; association/attachment/pairing of complementary strand; 2 max
(iii) annealing/splicing/backbones joined/phosphodiester bonds;
(b) (i) L and M ; 1
(ii) fragments 64 and 36(kilobases obtained) 1
59. (a) 1. DNA heated to 90 to $95^{\circ} \mathrm{C}$;
2. strands separate;
3. cooled / to temperature below $70^{\circ} \mathrm{C}$
4. primers bind;
5. nucleotides attach;
6. by complementary base pairing;
7. temperature $70-75^{\circ} \mathrm{C}$;
8. DNA polymerase joins nucleotides together;
9. cycle repeated;
(b) 1. percentage risk is too high for human application;
2. incorrect mRNA;
3. different tRNA/tRNA brings incorrect amino acid;
4. structure of protein synthesised unknown/sequence of amino acids changed/ incorrect shape/folding of polypeptide changed;
5. produce a toxic/harmful protein;
6. protein no
n-functional / chloride ions not transported / thick mucus results; 4 max
60. (a) Strand of DNA;

Short strand / up to 20 bases long;
With base sequence that is complementary to part of target gene;
Radioactive labelling / fluorescent labelling;
(b) Identify carrier (of cancer gene);

Identify which (cancer) gene present;
Identify most effective treatment;
61. (a) (i) Hydrolysis; 1
(ii) Shape / configuration complementary to (shape of) active site of enzyme;

Q Credit must not be awarded to answers that state the shapes are the same.
(iii) Consists of six antiparallel base pairs / six base pairs that read the same in opposite directions;
(b) (i) 3 ;
(ii) Partial digestion produced fragments of other lengths; e.g., $(3+2=5) /(4+1=5) /(4+2=6) /(3+2+1=6) /$ $(4+3=7) /(4+3+2=9)$;
(iii) 3 kb fragment is the smallest to be radioactive (so must be on left); 4 kb fragment is next smallest to be radioactive (so 1 kb fragment must be attached directly on to 3 kb fragment);

Q Credit should be given where answers show a clear understanding that the 3 kb and 4 kb fragments are the smallest to be radioactive and that the 4 kb fragment must be formed by the 3 kb and 1 kb fragments joined together.

