21.1

Separation of nucleic acid fragments by electrophoresis
Proteins are polymers of amino acids, which are different sizes and are charged molecules (1); as a result they would move through gel during electrophoresis under influence of an electric current in same way as nucleotides do – and they would similarly be separated by size and charge (1).

Pitfalls of profiling
1 11 loci was more than the earlier DNA tests and was affordable (1); producing a DNA profile now is increasingly faster and less expensive so a higher number of loci that are theoretically more sensitive and with less chance of misidentification is now affordable (1).
2 Siblings are different as a result of random gene assortment in gamete formation and random fusing of gametes (1); they share up to 100% of their DNA (1); although normally have different mixture of alleles, introns, etc (1); but by chance some siblings will have much more genetic similarity than others and could be seen as the same individual using 17 loci (1); cousins are less closely related, sharing at most 50% of their DNA, so chances of them having the same DNA profile is much reduced (1).

Summary questions
1 An intron is a large, non-coding (1); region of DNA (1); that is removed from messenger RNA before it is translated into a polypeptide chain (1).
2 Polymerase chain reaction (PCR) is a process by which a small piece of DNA is amplified/replicated many times (1); need relatively large DNA sample for DNA profile/sequencing (1); in forensic cases/criminal investigations samples available are often extremely small (1); PCR amplifies samples so DNA profiling can be carried out (1).
3 Benefits: can be used in criminal cases to prove guilt or innocence (1); tiny amounts of DNA can be used (1); DNA lasts a long time so ‘cold’ cases can be revived by DNA evidence (1); can be used to prove paternity / to prove or disprove familial relationships in immigration cases (1); can be used to identify species / can be used to find evolutionary relationships (1). Limitations: can be too dependent on it and ignore other evidence in criminal cases (1); DNA profiles can be done at different levels and mistakes can be made (1); contamination of samples with DNA from other organisms (1). Any other sensible points (6 max, up to 3 marks for benefits and up to 3 marks for limitations).
4 Variety of ways students might do this but must include: extract DNA from sample → PCR to amplify DNA if necessary → use restriction endonucleases to cut DNA sample into fragments at recognition sites → place fragments in wells of agarose gel electrophoresis plate with buffer, identifying fragments → pass electric current through plate so fragments move dependent on size and charge → transfer gel plate to alkaline buffer to denature DNA fragments → carry out Southern blotting by placing nylon filter of nitrocellulose paper over gel and using absorbent material to draw fluid through leaving DNA fragments on filter → fix fragments using UV light → add excess of radioactive or fluorescent gene probes to hybridise with fragments → wash off excess probes → use X-ray photography or UV light to show up bands of DNA profile (max 6).

21.2

Summary questions
1 DNA for sequencing is mixed with a primer, DNA polymerase, excess of normal nucleotides (bases A, T, C, and G) and terminator bases, each with a coloured fluorescent tag (1); → at optimum temperatures, DNA polymerase builds chains. Whenever a terminator base is incorporated, the chain is terminated and no more bases can be added (1); → process is repeated until all possible chains are created (1); → chains separated out by gel electrophoresis; (1) → lasers used to read fluorescent tags and learn order of bases in complementary DNA strand, from this original strand can be deciphered (1).
2a Original bacterial genome – around 548 days, high-throughput methods – under 1 day. Difference is 547 days (1).

b In original techniques each stage was carried out by hand in the laboratory (1); modern techniques mainly carried out in machines, many DNA fragments processed at once, so much faster and more efficient (1).

3a Bases that when added to a DNA chain during DNA synthesis terminate it / no more nucleotides can be added (1).

b Using terminator bases all possible length DNA fragments are synthesised (1); having different coloured fluorescent tags attached to four different terminator bases (1); makes it possible to work out sequence of original DNA (1); once chains have been separated using gel electrophoresis (1); high-throughput sequencing is much more complex and rapid (1), but still relies on terminator bases to terminate chains in final stages (1).

21.3

Synthetic life

Engineering new organisms to be used in biotechnology (1); engineering healthy genes to cure genetic diseases (1); engineering new viruses to destroy disease causing bacteria – any other sensible suggestions.

Infection outbreak – DNA Sequencing and clinical intervention

Benefits include: Ability to tell if an infection is bacterial or viral, and therefore whether to use antibiotics or not (1); ability to tell what type of bacterium is causing an infection and therefore use the right antibiotic to cure the infection and minimise risk of resistance developing (1); ability to pick out hospital based or community based infections (1); ability to develop right vaccine for new strain of flu etc (1); any other sensible points. Limits include: mainly cost (1); size of machines and expertise needed to prepare samples and interpret results (1).

Summary questions

1 Bioinformatics allows scientists to analyse large amounts of data generated during sequencing of billions of base pairs in genomes (1); display the data in ways that make sense and help identify patterns (1). Computational biology takes these results and uses them to build up models (1); e.g., of the spread of disease, the evolutionary relationships between organisms, the inheritance of antibiotic resistance in bacteria (1); and use them to model possibilities in different circumstances (1).

2a Each strain of a pathogen has a slightly different genome and so can be accurately identified by DNA sequencing (1); ability to identify a particular strain of a pathogen means country/place of origin can be identified (for treatment, quarantine, etc.) (1); or individuals with disease can be identified (for isolation, treatment, etc.) (1); spread of a strain of disease can be tracked and transmission methods understood to prevent further spread (1).

b Traditionally species identification was done by observation of anatomical and physiological features (1); with DNA sequencing genome similarities are examined and comparisons made to standard species genome (1); much more accurate but harder in the field (1); in understanding evolutionary relationships DNA sequencing looks at the difference in number of mutations between species (1); and by calculating average mutation rate (1); you can calculate when two species diverged (1).

3 Human genome sequencing shows there are 20–25 000 coding genes (1); proteomics suggests there are between 250 000 to 1 000 000 proteins (1); technology used to sequence DNA can also sequence amino acids in proteins – this is not always what you would predict from bases in DNA (1); some genes can code for up to 1000 different proteins (1); introns and some exons may be removed before mRNA lines up on ribosomes (1); spliceosomes combine exons in different orders to give different versions of mRNA (1); which code for different amino acid sequences and different proteins
(1); some proteins are modified by other proteins once they are formed (1); may remain intact, shortened or lengthened which creates other proteins (1).

21.4

Summary questions
1 Genetic engineering is the practical technique of isolating genes for desirable characteristics in one organism (1); and placing them into another organism (1); using a suitable vector (1).
2 **Restriction endonucleases** cut a required gene from DNA of an organism at specific sites within DNA molecule (1); may leave uneven ends and extended regions of unpaired bases are called sticky ends (1); used for inserting into DNA of a vector (1). **Reverse transcriptase enzymes** create DNA from mRNA used to make a particular protein (1); reverse of normal DNA transcription (1); synthesised DNA usually inserted into a vector same way as method using restriction endonucleases (1).
3 Plasmid cut open using same restriction endonucleases as used to excise the gene to be used (1); so sticky ends of DNA fragment/gene and cut plasmid are complementary/match (1); sticky ends lined up and DNA ligase joins them by forming phosphodiester bonds between the two strands of DNA (1); transgenic plasmids taken up by bacteria as a result of calcium ions and temperature making membrane permeable to plasmid or by electroporation when electric current makes membrane porous to plasmids (1); plasmids have gene for antibiotic resistance, so bacteria that take up engineered plasmids can be identified (1); plasmid also contains marker gene engineered in (site where plasmid is cut open) to show engineering has worked – usually fluorescence or enzyme which changes colour of medium – required gene is inserted in middle of marker – so bacteria showing marker trait are not successfully engineered (1).
4 a and b Flow diagrams should cover all main points. Each diagram can be awarded a maximum of 6 marks. Diagrams should include the points of difference in the processes, 4 marks should be reserved for the following points – without these a candidate cannot achieve full marks: infection of plant cell with bacteria containing engineered plasmid (1); callus formation (1); and growing on of cloned cells to form many individual transgenic plants (1); production of functional transgenic bacterium after plasmid is reabsorbed into host bacterial cell (1).

21.5

Summary questions
1 Genetically engineered microorganisms have been used safely for many years (1); genetically engineered microorganisms produce many beneficial materials from insulin to antibiotics so benefits are very tangible (1); little empathy for microorganisms and no welfare issues (1). Any other sensible points (max 3).
2 1 mark for each correct row

<table>
<thead>
<tr>
<th>Somatic cell gene therapy</th>
<th>Germ line cell therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>replacement of mutant/faulty gene in body cells with healthy/normal allele</td>
<td>replacement of mutant/faulty gene in egg, sperm or early embryo with healthy/normal allele</td>
</tr>
<tr>
<td>must be done in many cells</td>
<td>only needs to be done in one or two cells but required for every child born to an affected individual</td>
</tr>
<tr>
<td>have to get healthy allele into nucleus of cells</td>
<td>have to get healthy allele into nucleus of cells</td>
</tr>
<tr>
<td>gene needs to function long-term</td>
<td>gene needs to function long-term</td>
</tr>
<tr>
<td>healthy allele not passed on to offspring</td>
<td>healthy allele will function in individual and will be passed on to their children</td>
</tr>
<tr>
<td>does not cure the genetic disease</td>
<td>cures the genetic disease</td>
</tr>
</tbody>
</table>

3a Many potential benefits for example, pest-resistant GM crop varieties (1); soya beans reduce amount of pesticide spraying needed (1); protects other insects in the environment and helps poor
farmers who cannot afford to spray regularly (1); yield can be increased giving more food (1); weed killer resistant soya beans allow farmers to spray to get rid of weeds without affecting crop which then gets all the resources and so has bigger yield (1); other plants are engineered specifically for high yield (1); crops can grow in wider range of conditions/survive adverse conditions e.g., scuba rice developed by IRRI to help rice farmers cope with extreme weather events (1); extended shelf-life of some GM crops reduces food waste – either less food has to be produced or there is more food to go around (1); nutritional value of crops can be increased – enhanced levels of vitamin A in golden rice, enhanced protein, or carbohydrate in increased yields (1); plants used to grow medicines – vaccines in bananas, tomatoes, etc., antibiotics (1); disease resistant varieties can be reduced which reduces crop losses and provides more food. Any other sensible suggestions (1 mark for each potential benefit, max 6).

b Evidence of balanced discussion with different opinions considered. For example: Non-pest insects might be damaged by toxins in GM plants – for example Bt protein in modified soya plants could affect larvae of other moths and butterflies (1); antibiotic genes could spread from marker genes into wild populations and spread antibiotic resistance (1); transferred genes might mutate (1); biodiversity could be reduced if GM crops are herbicide resistant (1); people might be allergic to proteins grown in GM crops, e.g., Bt protein (1); insects may become resistant to pesticides in plant tissues. Two or more insecticide genes may be used to reduce chances of resistance developing (1).

4 Copy of human gene coding for relevant/desired protein is isolated or synthesised, introduced into genetic material of fertilised cow, sheep, or goat egg (1); promoter sequence added to ensure gene is expressed only in mammary glands (1); fertilised, (female) transgenic embryo is returned to mother to grow to birth. When mature transgenic animal conceives and gives birth, it produces milk containing desired human protein to be harvested (1); well-being of transgenic animals appears unaffected – simply make an extra protein in their milk (1); enormous benefits for human patients receiving transgenic proteins/pharmaceuticals, however there is ethical consideration of whether human genes should be put into animals (1); mother animal has to undergo fertility treatment to produce eggs and then embryos are placed in surrogate animals, which have to be medically-prepared (1); success rate of inserting human gene is low, so ova/embryos are wasted and destroyed (1); process involves germ line cell manipulation so genes are passed on in perpetuity (1). Any other sensible points (6 max).