Model Answers: Medium

1a

a) PCR was used because…

- Only small amounts of DNA were obtained (from each individual) **SO** PCR was needed to increase/amplify the amount/mass of DNA; [1 mark]
- So there is enough/sufficient DNA available for genetic fingerprinting; [1 mark]

[Total: 2 marks]

1b

b) i) DNA is heated to 94 °C because…

- To separate the two strands of the DNA **OR** to break the hydrogen bonds between the DNA strands/bases; [1 mark]

ii) DNA primers are…

- Short lengths/fragments of single-stranded DNA; [1 mark]
- Complementary to / to mark the beginning/ends of the part of DNA being amplified **OR** that are required for enzyme/DNA polymerase attachment to the nucleotides; [1 mark]

iii) Temperature stable enzymes are used because…

- So that enzymes do not denature / the bonds holding the tertiary structure of the enzyme in place do not break (at high temperatures/94 °C); [1 mark]

[Total: 4 marks]

For part i) you will not be awarded the mark for stating the DNA is 'unzipped' as this suggests that DNA helicase is being used.

1c

c) i) The father was…

- Adult C; [1 mark]

c) ii) The reason for this is that…

- (Adult C) is the only adult that can provide all the (remaining) DNA fragments/bands (after the mother's DNA is accounted for) that the children have **OR** all fragments/bands from adults B and C together/combined match all the children's fragments/bands; [1 mark]

[Total: 2 marks]

When talking about genetic fingerprinting you must refer to the DNA as 'fragments' or 'bands' you cannot call them 'genes'. This is because it has not been stated that the DNA was cut into separate genes and that the DNA could come from non-coding sections of the genome.

1d

d) Genetic fingerprinting is carried out as follows…

Any **five** of the following:

- DNA is extracted/isolated from a sample (e.g. cheek cell, root of a hair, blood, semen); [1 mark]
- DNA is copied/increased/amplified through PCR / the polymerase chain reaction; [1 mark]
- DNA sequences that are highly variable between individuals are selected; [1 mark]
- DNA is hydrolysed/cut into fragments using restriction enzymes/endonucleases; [1

mark]

- DNA fragments are separated using (gel) electrophoresis; [1 mark]
- (During gel electrophoresis the) DNA is put into wells in gel **AND** an electric current is passed through; [1 mark]
- Tracking dye used to show how far DNA fragments (from wells/samples) have travelled through the gel **OR** probe with fluorescent stain / radioactive marker added; [1 mark]
- (DNA) bands/fragments compared/matched with sample/reference DNA/DNA ladder (e.g. DNA from potential parent or suspected criminal); [1 mark]

[Total: 5 marks]

It is important in this topic for you to not only interpret the results of genetic techniques but also describe the **methods**. Method questions are usually high scorers and rely on relaying information that you can revise without any need for interpretation of data/context. It is therefore essential to dedicate time to learning the methods of all the techniques and remembering them step-by-step.

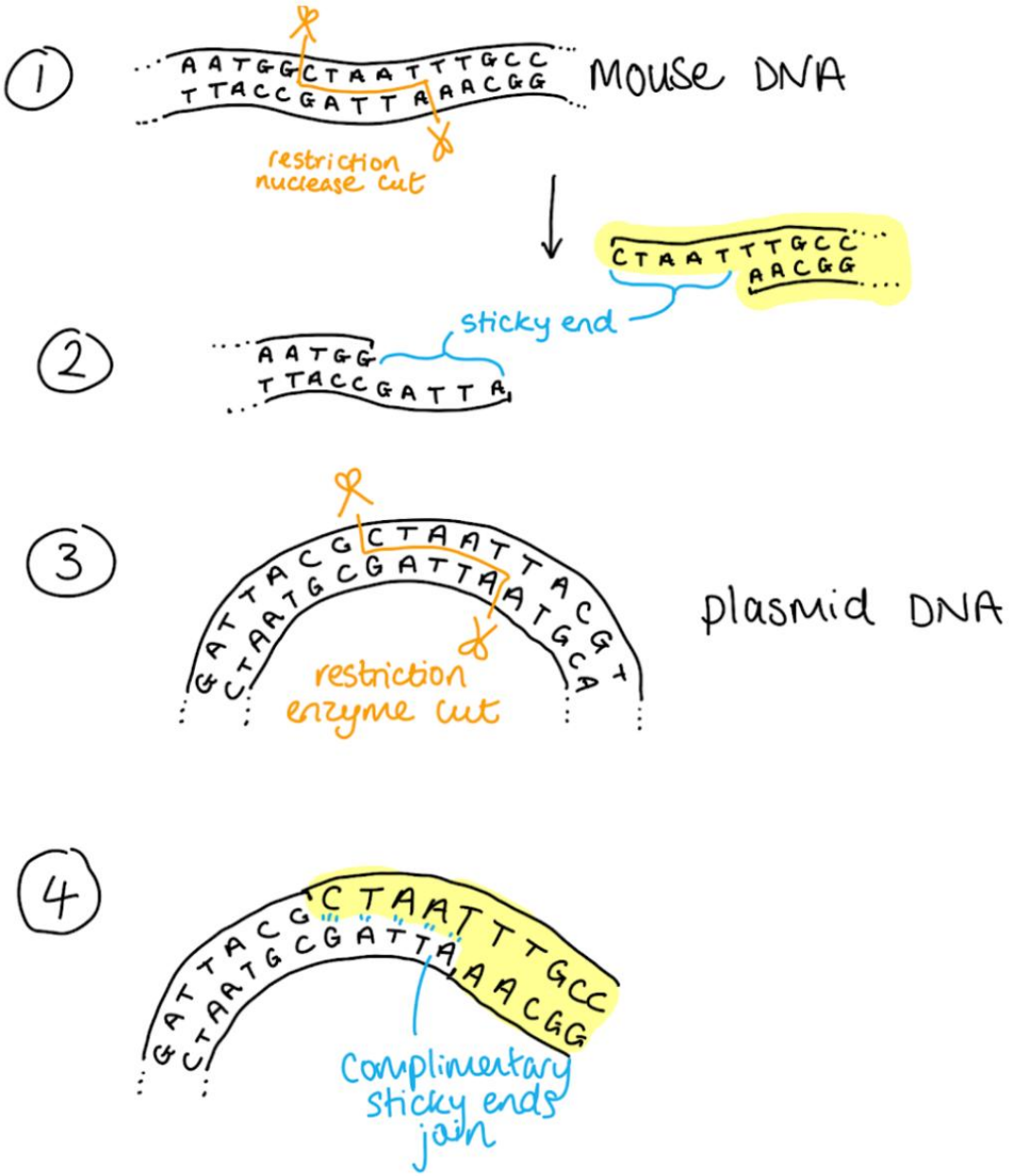
2a

a) The pieces of mouse DNA would be able to join the plasmid DNA because…

- Both mouse DNA and plasmid DNA have sticky ends **OR** both DNA ends have unpaired nucleotides ; [1 mark]
- The unpaired nucleotides / sticky ends are complementary **SO** base-pairing occurs; [1 mark]

[Total: 2 marks]

Take note of the underlined words in this mark scheme - restriction enzymes produce a staggered cut meaning that there is a short section of single-stranded DNA at the end of each resulting fragment. If the same enzyme is used to cut the mouse and plasmid DNA then the single-stranded sections will be complementary and form hydrogen bonds joining the separate fragments.



2b

b) i) The other enzyme added was...

- (DNA) ligase; [1 mark]

b) ii) The role of this enzyme is...

- To join the sugar-phosphate backbone / form phosphodiester bonds between DNA fragments and the plasmid DNA; [1 mark]

[Total: 2 marks]

Once the sticky ends have joined together and hydrogen bonds are formed spontaneously between complementary nucleotide bases, the DNA backbones are joined by DNA ligase.

2c

c) A vector is...

- A carrier of DNA / a gene; [1 mark]
- Into another cell/organism/host; [1 mark]

[Total: 2 marks]

2d

d) Human recombinant insulin can be produced as follows…

Any **six** of the following:

- Human insulin mRNA is extracted from human pancreatic cells; [1 mark]
- Reverse transcriptase is used to make complementary DNA/cDNA **OR** DNA is synthesised from nucleotides in the lab / in a synthesiser (machine); [1 mark]
- DNA polymerase is used to convert single-stranded cDNA into double-stranded cDNA; [1 mark]
- DNA/gene is amplified using PCR; [1 mark]
- Insulin gene/DNA is cut using restriction enzyme/endonuclease (to create sticky ends) **AND** bacterial plasmid cut using same restriction enzyme/endonuclease; [1 mark]
- Amplified gene is inserted into a plasmid (vector); [1 mark]
- The recombinant plasmid is introduced into host cells/bacteria; [1 mark]
- Transformed cells are identified using marker genes; [1 mark]
- Transformed cells are grown in culture / a fermenter; [1 mark]
- Expressed insulin is separated/extracted and purified from the host cells; [1 mark]

[Total: 6 marks]

The question has asked for the key steps in the commercial production of human insulin. Although improved methodologies have been developed (including using transgenic crops) these have not been implemented on a commercial scale. The standard robust process relies on isolating the gene of interest, inserting it into a plasmid/vector, inserting the plasmid into bacterial cells, and growing them on a large scale to ensure a large amount of insulin protein is produced/expressed.

Note that marks have not been awarded for points covered elsewhere in the question, i.e. sticky ends, ligase, and the role of vectors.

3a

a) The zebrafish is attached to protein Q because…

- It acts as a marker gene to show that the pig gene has been taken up / that cells have been transformed; [1 mark]
- Only cells/embryos that contain the gene will (express the gene and) fluoresce / give off blue light; [1 mark]

[Total: 2 marks]

Marker genes are used to verify that the gene has been taken up by the cells and the protein is being expressed. Remember it is the **protein** that fluoresces blue, **not the gene**. This can sometimes act as a way of selecting cells that express the protein.

3b

b) i) The role of a promoter is…

- It is the region of a gene to which RNA polymerase/transcription factors bind to initiate/start transcription; [1 mark]

b) ii) Goat promoter DNA from a gene normally expressed in the milk glands is used because…

- The gene is only expressed in the milk glands / udder cells **OR** the gene is not

expressed in every cell in the goat; [1 mark]

[Total: 2 marks]

The protein is expressed in milk, so it does not enter the bloodstream and is easy to harvest in the milk; both of these features make this a safe way of producing protein Q.

3c

c) i) During gel electrophoresis DNA moves towards…

- The anode / positive (+ve) electrode; [1 mark]

c) ii) The reason for this is…

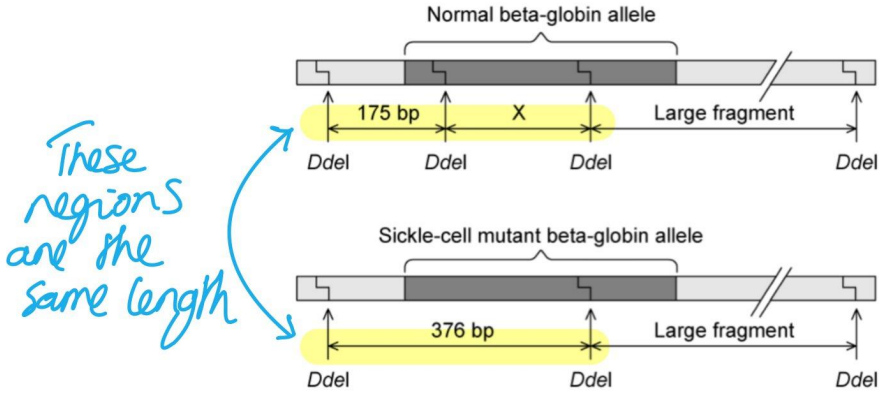
- DNA is negatively charged; [1 mark]
- Due to the phosphate groups (in the phosphate backbone); [1 mark]

[Total: 3 marks]

3d

d) i) Fragment **X** is…

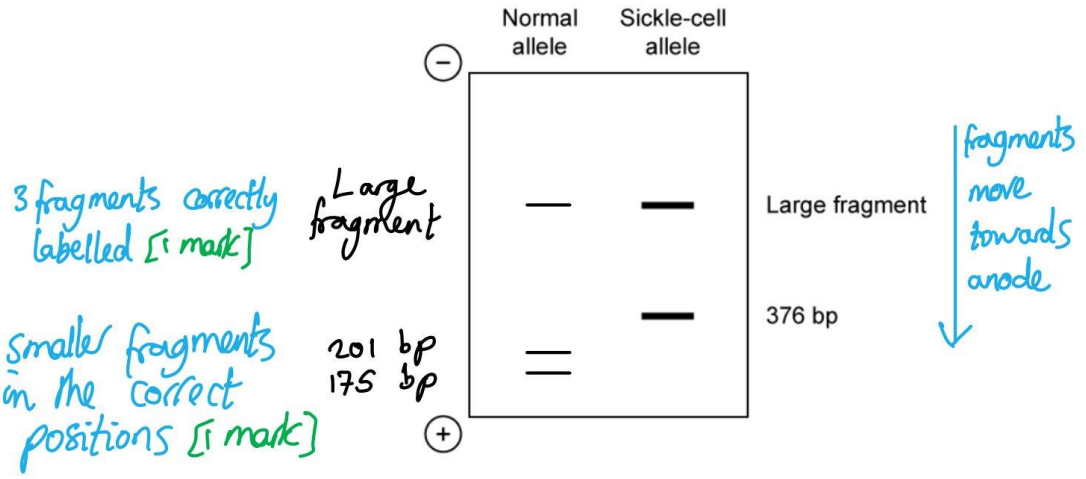
- 201 bp in length; [1 mark]



Fragment X = 376 − 175 [1 mark]
= 201 bp [1 mark]

d) ii) The following fragments should be drawn…

- A large fragment in the same position as the large fragment in the sickle cell allele **AND** a fragment labelled 175 bp **AND** a fragment labelled 201 bp; [1 mark]
- The fragments labelled 175 bp and 201 bp have both travelled further than the 376 bp fragments in the sickle cell allele **AND** the fragment labelled 175 bp has travelled furthest; [1 mark]



[Total: 3 marks]

The smaller restriction fragments are 201 bp and 175 bp in length so will migrate further down the gel compared to the 376 bp fragment as they are smaller and have less mass.

4a

a) The role of DNA polymerase in RT-PCR is to...

- Join nucleotides to each other / form phosphodiester bonds between nucleotides / form the new sugar-phosphate backbone; [1 mark]

The role of reverse transcriptase in RT-PCR is to...

- Produce cDNA/complementary DNA from mRNA; [1 mark]

Reject *any answer that suggests DNA polymerase is involved with the formation of hydrogen bonds or complementary base pairing.*

[Total: 2 marks]

It is essential that you know the roles of DNA polymerase, reverse transcriptase, and ligase within gene technologies. Not only will you be asked about their roles in questions but they also give good hints about what is happening in a particular technique and can help you if you don't know the technique itself.

4b

b) The DNA is hydrolysed because...

- Any DNA present would be amplified/replicated **OR** the researcher only wanted the RNA present to be amplified; [1 mark]

[Total: 1 mark]

PCR does not discriminate between what you want to amplify and what you do not and therefore if you only want the RNA present to be amplified then the DNA must be destroyed. If the DNA is not destroyed then the whole genome could be amplified and this will not give an accurate representation of the genes that the cell is currently expressing.

4c

c) DNA replication stops because···

Any **one** of the following:

- DNA polymerase (eventually) denatures; [1 mark]

- There is a limited number of primers/nucleotides and / the primers/nucleotides are used up; [1 mark]

[Total: 1 mark]

The polymerase chain reaction is a cyclical process that uses temperature changes to amplify a DNA sample through semi-conservative replication. It requires primers to initially anneal the DNA fragments and act as a starting point for DNA polymerase to add free nucleotides. Therefore the lack of either primers, nucleotides, or DNA polymerase will stop replication.

4d

d) The researchers produced a variety of primers because…

- Base sequences differ/vary (between different viruses in the family); [1 mark]
- Therefore different complementary primers required **OR** primers with different complementary base sequences are required (to bind to the DNA of different viruses); [1 mark]

[Total: 2 marks]

Primers are lengths of complementary DNA that anneal to sections of DNA to aid in replication.