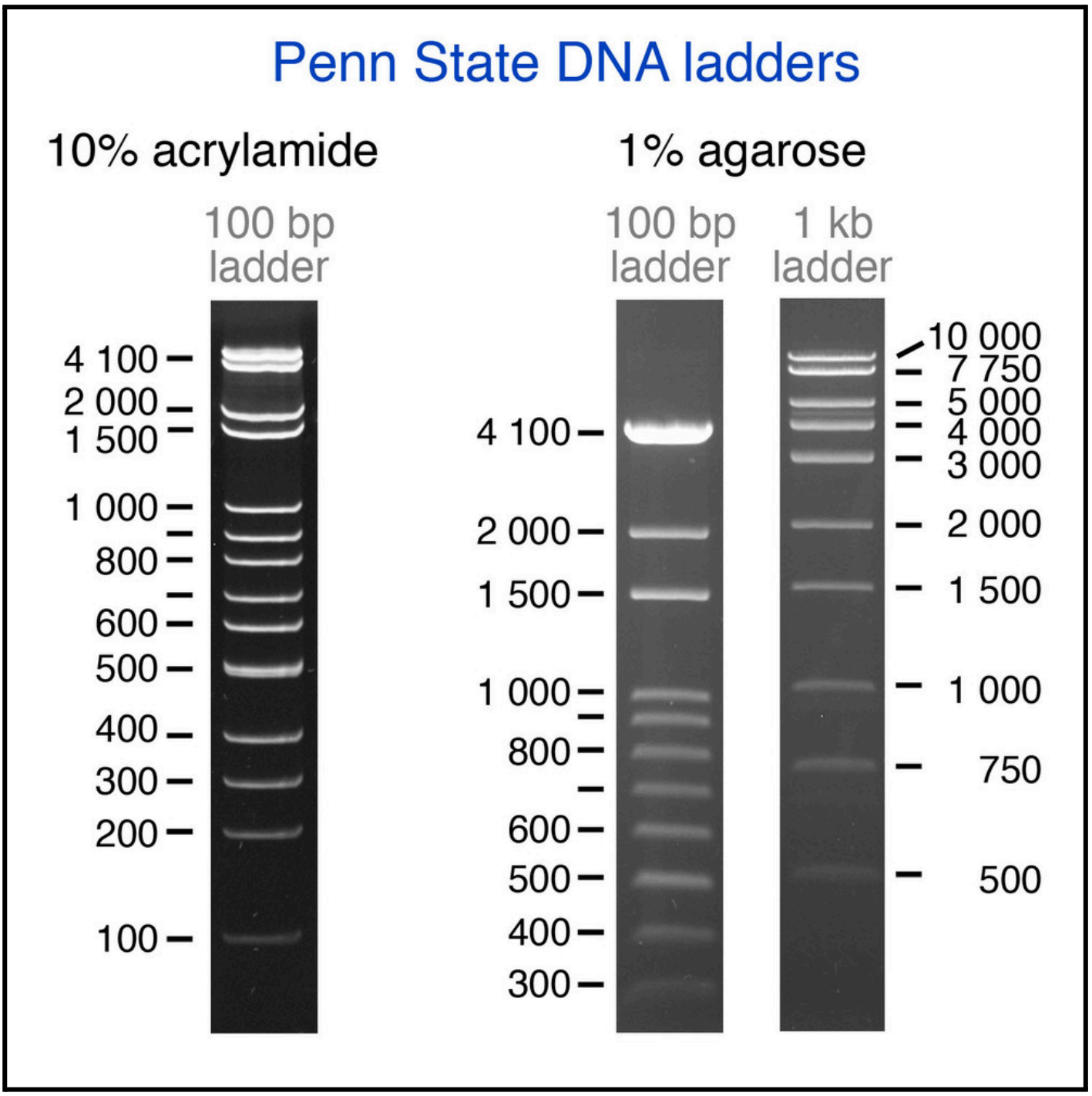
A Level Biology.

Electrophoresis & Gene Probes Practice.

Name :



Q1.Some populations of flies are becoming resistant to insecticides intended to kill them.

Scientists developed a method for finding out whether a fly was carrying a recessive allele, r, that gives resistance to an insecticide. The dominant allele, R, of this gene does not give resistance.

The scientists:

- **crossed flies with genotype RR with flies with genotype rr**
- **obtained DNA samples from the parents and offspring**
- **used the same restriction endonuclease enzymes on each sample, to obtain DNA fragments.**

(a) Explain why the scientists used the same restriction endonuclease enzymes on each DNA sample.

(2)

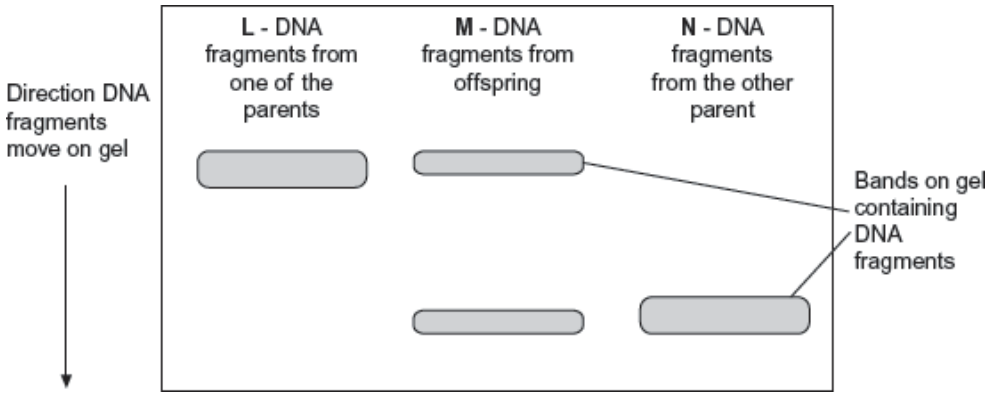
The scientists added two different primers to each sample of DNA fragments for the polymerase chain reaction (PCR).

- **Primer A3 only binds to a 195 base-pair fragment from allele r.**
- **Primer A4 only binds to a 135 base-pair fragment from allele R.**

The scientists separated the DNA fragments produced by the PCR on a gel where shorter fragments move further in a given time.

Their results are shown in Figure 1.

Figure 1



(b) Explain why primer A3 and primer A4 only bind to specific DNA fragments.

(2)

(c) Use all the information given to explain the results in Figure 1.

(3)

(d) The scientists wanted to know on which chromosome the gene with alleles R and r was located. From the flies with genotype RR, they obtained cells that were in mitosis and added a labelled DNA probe specific for allele R. They then looked at the cells under an optical microscope.

Explain why they used cells that were in mitosis.

(2)

Q2.

Scientists wanted to measure how much mRNA was transcribed from allele A of a gene in a sample of cells. This gene exists in two forms, A and a.

The scientists isolated mRNA from the cells. They added an enzyme to mRNA to produce cDNA.

(a) Name the type of enzyme used to produce the cDNA.

(1)

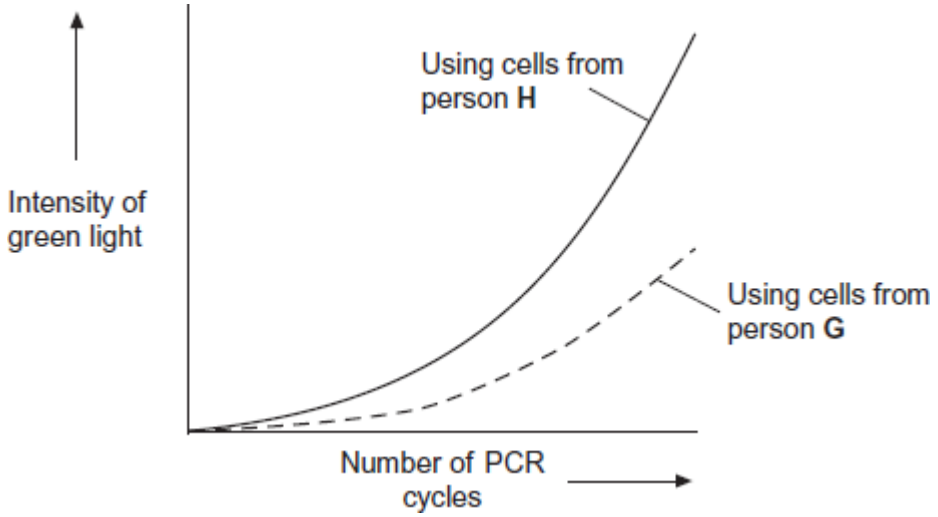
The scientists used the polymerase chain reaction (PCR) to produce copies of the cDNA. They added a DNA probe for allele A to the cDNA copies. This DNA probe had a dye attached to it. This dye glows with a green light only when the DNA probe is attached to its target cDNA.

(b) Explain why this DNA probe will only detect allele A.

(2)

(c) The scientists used this method with cells from two people, H and G.
One person was homozygous, AA, and the other was heterozygous, Aa.
The scientists used the PCR and the DNA probe specific for allele A on the cDNA from both people.

The figure shows the scientists' results.



(i) Explain the curve for person H.

(3)

(ii) Which person, H or G, was heterozygous, Aa? Explain your answer.

(2)
(Total 8 marks)

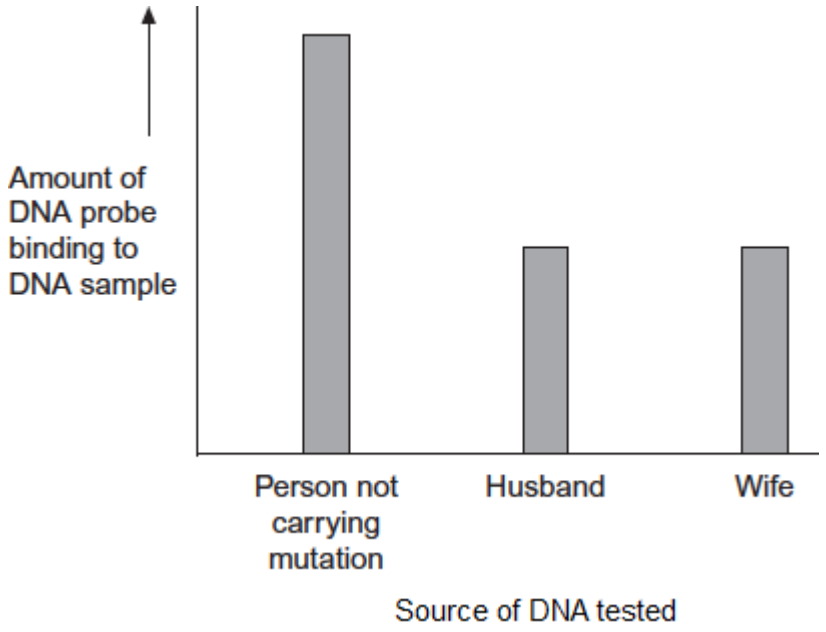
Q3.

A husband and wife wanted to know whether they were carriers of the mutated form of a gene. This mutation is a deletion that causes a serious inherited genetic disorder in people who are homozygous.

A geneticist took samples of DNA from the husband and the wife. He used a DNA probe to look for the deletion mutation. The DNA probe was specific to a particular base sequence in an exon in the gene. Exons are the coding sequences in a gene.

The geneticist compared the couple's DNA with that of a person known not to carry this mutation.

The chart shows the geneticist's results.



(a) The geneticist told the couple they were both carriers of the mutated gene.
Explain how he reached this conclusion.

(3)

(b) The DNA probe the geneticist used was for an exon in the DNA, not an intron. Explain why.

(3)

(c) To make the DNA probe, the geneticist had to find the base sequence of the normal gene. Once he had copies of the gene, what methods would he use to find the base sequence of the gene?

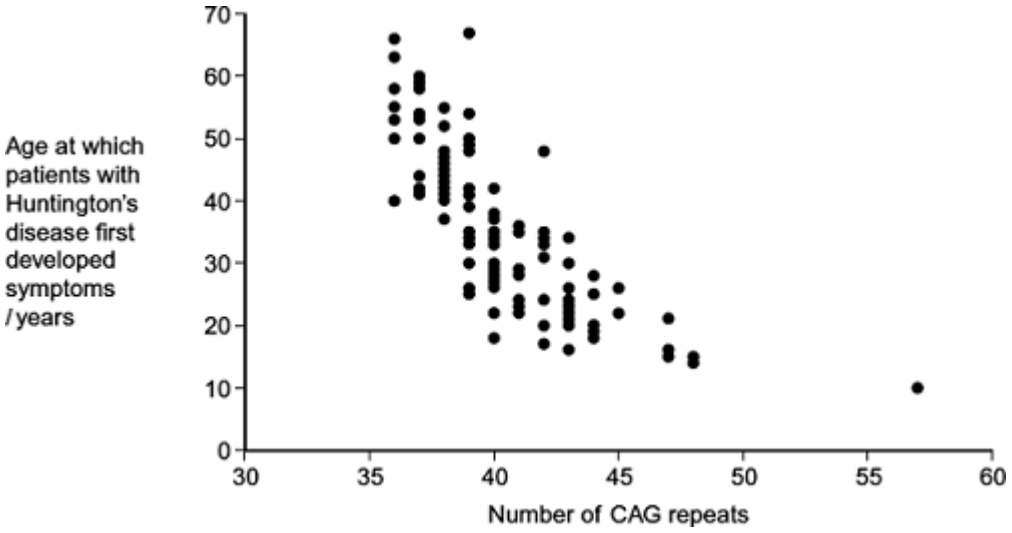
(2)
(Total 8 marks)

Q4.

Huntington's disease is a genetic condition that leads to a loss in brain function. The gene involved contains a section of DNA with many repeats of the base sequence CAG. The number of these repeats determines whether or not an allele of this gene will cause Huntington's disease.

- **An allele with 40 or more CAG repeats will cause Huntington's disease.**
- **An allele with 36 – 39 CAG repeats may cause Huntington's disease.**
- **An allele with fewer than 36 CAG repeats will not cause Huntington's disease.**

The graph shows the age at which a sample of patients with Huntington's disease first developed symptoms and the number of CAG repeats in the allele causing Huntington's disease in each patient.



(a) (i) People can be tested to see whether they have an allele for this gene with more than 36 CAG repeats. Some doctors suggest that the results can be used to predict the age at which someone will develop Huntington's disease.

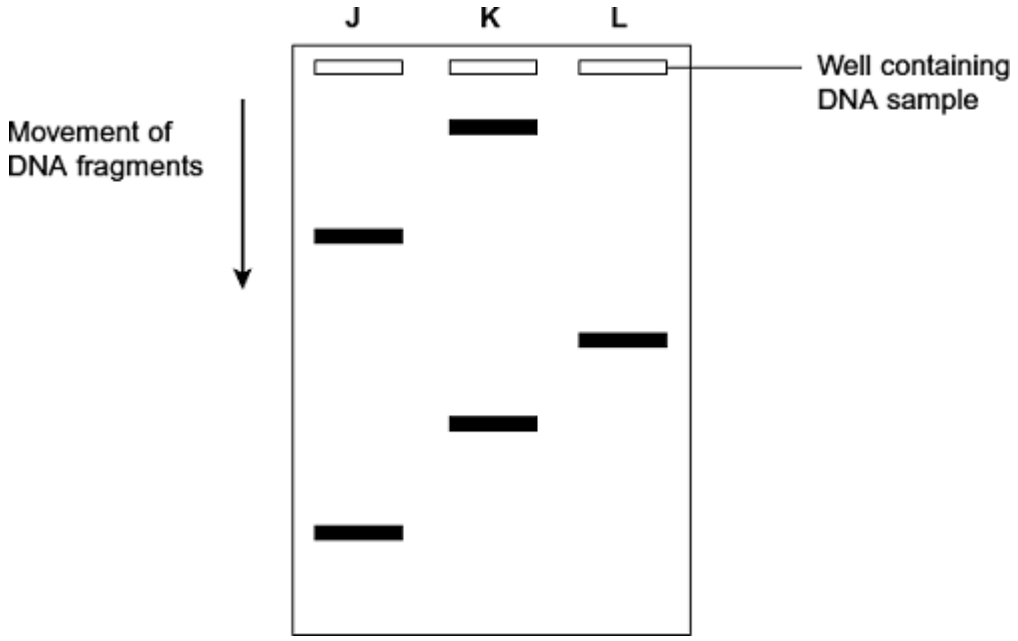
Use information in the graph to evaluate this suggestion.

(3)

(ii) Huntington's disease is always fatal. Despite this, the allele is passed on in human populations. Use information in the graph to suggest why.

(2)

(b) Scientists took DNA samples from three people, J, K and L. They used the polymerase chain reaction (PCR) to produce many copies of the piece of DNA containing the CAG repeats obtained from each person. They separated the DNA fragments by gel electrophoresis. A radioactively labelled probe was then used to detect the fragments. The diagram shows the appearance of part of the gel after an X-ray was taken. The bands show the DNA fragments that contain the CAG repeats.



(i) Only one of these people tested positive for Huntington's disease. Which person was this? Explain your answer.

Person
Reason

(2)

(ii) The diagram only shows part of the gel. Suggest how the scientists found the number of CAG repeats in the bands shown on the gel.

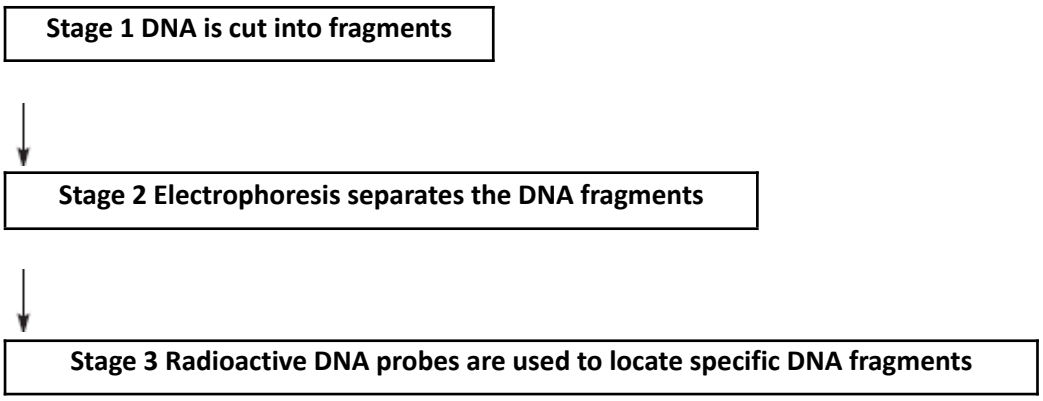
(1)

(iii) Two bands are usually seen for each person tested. Suggest why only one band was seen for Person L.

(1)

(Total 9 marks)

Q8. DNA probes may be used to identify the presence of specific genes associated with human diseases. The flow chart summarises the way in which they are used.



(a) Name the enzyme used in Stage 1.

(1)

(b) Explain how electrophoresis separates the fragments of DNA in Stage 2.

(2)

(c) (i) What is a *DNA probe*?

(2)

(ii) Explain why *radioactive* DNA probes are used to locate specific DNA fragments.

(2)

(Total 7 marks)

Q9.

A gene was broken into fragments using enzyme Z. The mixture of fragments produced was then separated by electrophoresis.

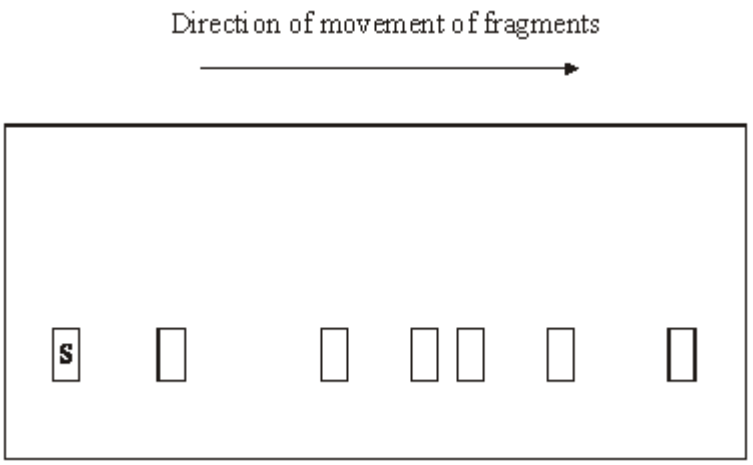
(a) What type of enzyme is enzyme Z?

(1)

The table shows the number of base pairs present in the fragments.

Fragment	Number of base pairs ($\times 10^3$)
1	4.65
2	5.72
3	10.71
4	2.39
5	5.35
6	7.53

The diagram shows the electrophoresis gel used. The mixture of fragments was placed at the start point marked S and the process started. The boxes indicate the positions reached by the different fragments.



(b) Explain why base pairs are a suitable way of measuring the length of a piece of DNA.

(2)

(c) (i) Write 6 above the appropriate box on the diagram to show the position you would expect fragment 6 to have reached.

(1)

(ii) Explain how you arrived at your answer.

(1)

(d) Enzyme Z recognises a particular sequence of bases in the gene. How many times does this sequence appear in the DNA of this gene?

(1)

(Total 6 marks)

Q10.

β-thalassaemia is a genetic condition in which abnormal haemoglobin is produced. In one form, the recessive allele for β-thalassaemia, t, differs from the normal allele, T, by a single base-pair. A radioactive DNA probe was used to investigate the genotypes of four members of one family. The flowchart summarises the technique involved.

DNA samples extracted and cut into fragments using a restriction enzyme

↓

Fragments separated from each other by electrophoresis

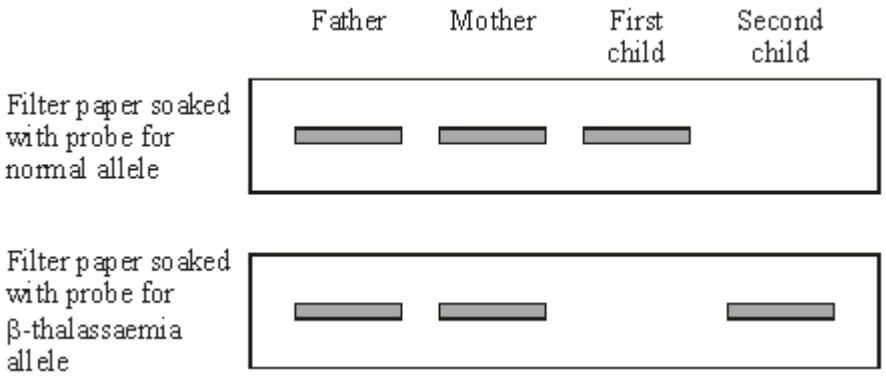
↓

One region of the resulting gel was blotted with two pieces of filter paper. The first was soaked in a solution containing a radioactive DNA probe for the normal allele.
The second was soaked in a solution containing a radioactive DNA probe for the β-thalassaemia allele.

↓

Surplus probe washed off

The diagram below shows the appearance of the two pieces of filter paper which resulted from the investigation.



(a) What is the probability that the next child that this couple have is a girl who has β-thalassaemia? Explain your answer.

(3)

(b) (i) The fragment of DNA containing the normal allele and the fragment with the β-thalassaemia allele moved the same distance on the gel. Explain why.

(2)

(ii) The allele for β-thalassaemia differs from the normal allele by only one base-pair. Explain why the probe used to identify these alleles consists of a piece of DNA twenty bases in length and not just one base.

(2)

(Total 7 marks)

Q11. Read the following passage.

Shark-fin soup is an expensive delicacy. To provide the basic ingredient, fishermen catch the sharks, hack the fins off and throw the dead bodies back into the ocean. But sharks are slow to mature and produce only a few offspring at a time, so they are vulnerable to overfishing. Monitoring the shark-fin trade is difficult, as once a fin has been cut off, it can be extremely difficult to work out precisely from which species it was taken.

The DNA from different species of sharks shows some differences in base sequence. This has enabled a new genetic fingerprinting technique to be developed. This technique would allow conservationists and fisheries managers to assess which of the 400 shark species are most threatened by the trade in shark fins.

An identification process has been developed using a range of "primers". These are short pieces of single-stranded DNA that are complementary to a particular sequence of DNA. Each primer is specific to the DNA of one shark species.

The primers are added to DNA taken from a shark's fin and the polymerase chain reaction is carried out. Only two primers, one at each end of a certain piece of DNA, will bind. The piece of DNA between the primers is replicated by the polymerase chain reaction. The primers that bind are specific to a particular species of shark and the length of the DNA fragment replicated differs for each species. When this DNA is run in an electrophoresis gel it produces a single band, enabling the researchers to identify which species of shark is involved.

Use information from the passage and your own knowledge to answer the questions.

(a) (i) Explain why the DNA for each species of shark shows differences in base sequence (line 6).

(2)

(ii) Each primer is specific to the DNA of one shark species (line 12).

Explain why a particular primer will only bind to the DNA of one species.

(2)

(iii) The length of the replicated DNA fragment is different for each species.

Explain why this is important in identifying the shark species involved.

(3)

(b) In conventional DNA fingerprinting, a series of bands is produced on the electrophoresis gel, resembling the rungs of a ladder. When the DNA in this new genetic fingerprinting technique is run in an electrophoresis gel it produces just one of these 'rungs'.

Explain the reason for the difference in the number of 'rungs' produced.

(2)

(c) Describe the polymerase chain reaction.

(6)

(Total 15 marks)

Mark Scheme.

M1.

(a)

1. Cut (DNA) at same (base) sequence / (recognition) sequence;

Accept: cut DNA at same place

2. (So) get (fragments with gene) R / required gene.

Accept: 'allele' for 'gene' / same gene

2

(b)

1. Each has / they have a specific base sequence;
2. That is complementary (to allele r or R).

Accept description of 'complementary'

2

(c)

1. Fragments L from parent rr, because all longer fragments / 195 base pair fragments;

Ignore: references to fragments that move further / less, require identification of longer / shorter or 195 / 135

Accept: (homozygous) recessive

2. Fragments N from parent RR, because all shorter fragments / 135 base pair fragments;

1 and 2 Accept: A3 for 195 and A4 for 135

2. Accept: (homozygous) dominant

3. (M from) offspring heterozygous / Rr / have both 195 and 135 base pair fragments.

Accept: have both bands / strips

Reject: primer longer / shorter

3

(d)

1. (Cells in mitosis) chromosomes visible;
2. (So) can see which chromosome DNA probe attached to.

2

(e) (i)

1. For comparison with resistant flies / other (two) experiments / groups;

Ignore: compare results / data / no other factors

2. To see death rate (in non-resistant) / to see effect of insecticide in non-resistant / normal flies.

Accept: 'pesticide' as 'insecticide'

Accept to see that insecticide worked / to see effect of enzyme

2

(ii) (PM must be involved because)

1. **Few resistant flies die (without inhibitor);**
2. **More inhibited flies die than resistant flies;**
3. **(PM) inhibited flies die faster (than resistant flies);**

(Other factors must be involved because)

4. **Some resistant flies die;**
5. **But (with inhibitor) still have greater resistance / die slower than non-resistant flies.**

Accept: (with inhibitor) die slower than non-resistant flies

4 max

[15]

M2.

(a) Reverse transcriptase;

1

(b)

1. Probe (base sequence) complementary (to DNA of allele A / where A is (and) binds by forming base pairs / hydrogen bonds;

Accept gene A

2. So (only) this DNA labelled / has green dye / gives out (green) light;

Accept glows for green light

2

(c) (i)

1. More probe binding / more cDNA / mRNA / more allele / gene A means more light;

2. DNA (with A) doubles each (PCR) cycle;

3. So light (approximately) doubles / curve steepens more and more (each cycle) / curve goes up exponentially / increases even faster;

3

(ii) (G because)

1. (Heterozygous) only has half the amount of probe for A attaching / only half the amount of DNA / allele A (to bind to);

Accept only one A to bind to

2. (So,) only produced (about) half the light / glow / intensity (of H) (per cycle of PCR);

If reference to 'half' for point 1, allow 'less light' in 2.

2

[8]

M3.(a)

1. Carriers are heterozygous / have one normal copy and one mutant copy of gene / have one recessive allele / don't have the condition;

2. Both have DNA that binds (about) half / 50% amount of probe (that non-carrier does);

3. Probe binds to dominant / healthy allele so only one copy of exon in their DNA / have one copy of gene without exon / base sequence for probe to bind to;

3. Accept normal and gene

3. Accept have a deletion mutation

3

(b)

1. Introns not translated / not in mRNA / (exons) code for amino acids / introns do not code for amino acids;

1. Accept not expressed

1. Accept polypeptide / protein for amino acids

2. Mutations of these (exons) affect amino acid sequences (that produce) faulty protein / change tertiary structure of protein;

2. Accept deletion leads to frameshift

2. In this context, accept affects protein made

3. So important to know if parents' exons affected, rather than any other part of DNA / introns;

Accept converse arguments involving - eg introns do not code for amino acids / proteins

Reject references to making amino acids, once

3

(c)

1. Restriction mapping / described;

2. DNA / base sequencing (of fragments) / description / name of method;

2

[8]

M4.

(a) (i)

1. Negative correlation;

Accept: description for 'negative correlation'
Neutral: 'correlation'
Reject: positive correlation

2. Wide range;

3. Overlap;

4. (Graph suggests that) other factors may be involved (in age of onset);

2 / 3 Accept the use of figures from the graph
2 / 3 Can refer to age of onset or number of CAG repeats
Ignore references to methodology

3 max

(ii)

1. Age of onset can be high / symptoms appear later in life;

Accept: 'gene' for 'allele'

2. (So) individuals have already had children / allele has been passed on;

OR

3. Individuals have passed on the allele / already had children;

4. Before symptoms occur;

2 max

(b) (i)

1. Person K;

2. (As has) high(est) band / band that travelled a short(est) distance / (er) so has large(st) fragment / number of CAG repeats;

Must correctly link distance moved and fragment size

2

(ii) Run fragments of known length / CAG repeats (at the same time);

Accept: references to a DNA ladder / DNA markers
Do not accept DNA sequencing

(iii) Homozygous / (CAG) fragments are the same length / size / mass;

Accept: small fragment has run off gel / travelled further

1

[9]

M8.

(a) Restriction (enzyme / endonuclease);

1

(b) Move towards anode / move because charged;

Different rates of movement related to charge / size;

2

(c)

(i) Piece of DNA;
Single stranded;
Complementary to / binds to known base sequence / gene;

max 2

(ii) DNA invisible on gel / membrane;
Allows detection;

2

[7]

M9.

(a) Endonuclease / restriction enzyme;

1

(b) DNA made of base pairs;
Each base pair is same length / occupies same distance along backbone;

2

(c)

(i) Second blank box from left labelled 6;

1

(ii) Distance moved depends on length / number of base pairs / second longest fragment / second shortest distance identified;

1

(d) 5;

1

[6]

M10.

(a) Mother and father both heterozygotes / Tt / carriers;
Probability of thalassaemia 1/4 and female 1/2;
Probability of both 1/8;

3

(b) (i) Cut at same base sequence as same enzyme used;
Fragments are same length / size / have same charge;

2

(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.

2

[7]

M11.

(a) (i) Different genes / characteristics / features;
Reference to mutations;
Or
Base sequence determines protein;
Different species have different protein sequences;

max 2

(ii) Primer has different DNA sequence;
DNA specific / complementary base-pairing;

2

(iii) Electrophoresis separates DNA;
(So they can be) identified by position on gel;
Smaller / shortest fragments travel furthest / quicker / or reverse argument;

3

(b) (*conventional*) Many lengths / all DNA / (*new*) one length;
Each rung is DNA of one / specific length;

2

(c) 1 Heat DNA;
2 Breaks hydrogen bonds / separates strands;
3 Add primers;
4 Add nucleotides;
5 Cool;
6 (to allow) binding of nucleotides / primers;
7 DNA polymerase;
8 Role of (DNA) polymerase;
9 Repeat cycle many times;

max 6

[15]