**Q1.** Read the following passage.

In 1991, nine skeletons were found in Russia. They were believed to be those of Tsar Nicholas II, his family and staff who were killed in 1917 during the Russian revolution. Very small amounts of DNA were isolated from these skeletons. This DNA was used in the polymerase chain reaction (PCR). Genetic fingerprinting was then carried out on this DNA to identify the skeletons.

The chart shows some of the results obtained from the genetic fingerprinting of seven of the skeletons, three children and four adults.



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

(a)     Explain why the polymerase chain reaction was used in this investigation.

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**(2)**

(b)     In the polymerase chain reaction, DNA is heated to 95 °C and nucleotides, enzymes and DNA primers are added to the mixture.

(i)      Explain why the DNA is heated to 95 °C.

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**(1)**

(ii)     What are DNA *primers*?

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**(1)**

(iii)     Why are DNA primers added during the polymerase chain reaction?

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**(1)**

(iv)    What is the advantage of the enzyme used in the polymerase chain reaction being thermostable?

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**(2)**

(c)     Describe how genetic fingerprinting is carried out.

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**(6)**

(d)     All three children on the chart had the same parents. One of the parents was **Adult 1**.

Which of the other three adults on the chart was the other parent? Give the reason for your answer.

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**(2)**

**(Total 15 marks)**

 **Q2.**          The polymerase chain reaction is a process which can be carried out in a laboratory to replicate DNA. The diagram shows the main stages involved in the polymerase chain reaction.



(a)     Explain why DNA is heated to 95 °C.

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**(1)**

(b)     What is the role of

(i)      a primer in this process;

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**(1)**

(ii)     DNA polymerase?

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**(1)**

(c)     (i)      How many DNA molecules will have been produced from one molecule of DNA after 6 complete cycles?

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**(1)**

(ii)     Suggest **one** use of the polymerase chain reaction.

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**(1)**

(d)     Give **two** ways in which the polymerase chain reaction differs from the process of transcription.

1 ...................................................................................................................

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2 ...................................................................................................................

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**(2)**

**(Total 7 marks)**

**Q3.**          (a)     Explain the reason for each of the following in the polymerase chain reaction (PCR).

(i)      DNA is heated to 95 °C.

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**(1)**

(ii)     DNA polymerase used is heat-stable.

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**(1)**

(iii)     The reaction mixture is cooled to 40 °C.

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**(1)**

(b)     The graph shows the number of DNA molecules made using PCR, starting with one molecule.



(i)      Explain the shape of the curve from cycles 1 to 16.

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**(2)**

(ii)     Suggest **one** explanation for the levelling out of the curve from cycles 17 to 20.

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**(2)**

**(Total 7 marks)**

**Q4.**         The polymerase chain reaction (PCR) can be used to produce large quantities of DNA.
Describe how the PCR is carried out.

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**(Total 6 marks)**

**Q5.**          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.

10   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).

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**(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.

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**(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.

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**(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.

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**(2)**

(c)     Describe the polymerase chain reaction.

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**(6)**

**(Total 15 marks)**

**Q6.**          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**?

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**(1)**

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

|  |  |
| --- | --- |
| **Fragment** | **Number of base pairs (× 103)** |
| 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.

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**(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.

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**(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?

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**(1)**

**(Total 6 marks)**

**Q7.**          Read the following passage.

DNA tests were used to confirm the identity of deposed Iraqi leader Saddam Hussein, after his capture in December 2003. DNA tests were carried out to prove the suspect was not one of the many alleged “look alikes” of the former leader.

Firstly, the DNA was extracted from the mouth of the captured man using a swab. Great care was taken to check that the swab did not become contaminated with any other DNA. DNA extracted from the swab was then subjected to a standard technique called the polymerase chain reaction (PCR), which takes a couple of hours. Lastly, the sample was “typed” to give the genetic fingerprint. This was produced within 24 hours of Saddam Hussein’s capture.

Tests for use in criminal cases often take much longer because samples are very small or

10      contaminated.

It appears that Hussein’s genetic fingerprint was already stored away for comparison. This was obtained from personal items such as his toothbrush. DNA from the toothbrush would have been subjected to PCR before a DNA fingerprint could have been obtained.

*Source:* adapted from SHAONI BHATTACHARYA, *New Scientist* 15 December, 2003

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

(a)     Describe how the technique of genetic fingerprinting is carried out and explain how it can be used to identify a person, such as Saddam Hussein.

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**(6)**

(b)     Explain how DNA could be present on a toothbrush (line 12).

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**(2)**

(c)     (i)      Explain why the polymerase chain reaction was used on the sample of DNA from the toothbrush (lines 12-13).

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**(2)**

(ii)     Explain **one** way in which the polymerase chain reaction differs from DNA replication in a cell.

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**(2)**

(d)     Tests for use in criminal cases often take much longer because samples are very small or contaminated (lines 8-10). Explain why it takes longer to obtain a genetic fingerprint if the sample is

(i)      very small;

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**(1)**

(ii)     contaminated.

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**(2)**

**(Total 15 marks)**

**Q8.**          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 belowshows 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.

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**(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.

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**(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.

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**(2)**

**(Total 7 marks)**

**Q9.**          Read the following passage.

The giant panda is one of the rarest animals in the world and is considered to be on the brink of extinction in the wild. Giant pandas have been kept and bred in zoos with the hope that they could be released into the wild. One worry is that small populations, like those in zoos, reduce the genetic variation needed to allow the species to

5        adapt to changing conditions. Unfortunately, pandas find it difficult to reproduce in captivity. Fertilisation of the females is guaranteed only by insemination with semen from several males. With so many potential fathers, the true paternity of the cubs is not clear. It is important to identify the fathers to maintain genetic variation.

10      Panda faeces can be collected in the wild. The faeces contain DNA from the panda, from the bamboo on which they feed and from bacteria. The DNA is subjected to the polymerase chain reaction (PCR). The primers used attach only to the panda DNA. The resulting DNA is subjected to genetic fingerprinting. This can help us to count the number of individuals in the wild because it allows us to identify individual pandas.

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

(a)     Describe how genetic fingerprinting may be carried out on a sample of panda DNA.

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**(6)**

(b)     (i)      Explain how genetic fingerprinting allows scientists to identify the father of a particular panda cub.

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**(2)**

(ii)     When pandas are bred in zoos, it is important to ensure only unrelated pandas breed. Suggest how genetic fingerprints might be used to do this.

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**(1)**

(c)     (i)      Suggest why panda DNA is found in faeces. (line 10)

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**(1)**

(ii)     Explain why the PCR is carried out on the DNA from the faeces. (line 12)

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**(1)**

(iii)     Explain why the primers used in the PCR will bind to panda DNA, but not to DNA from bacteria or bamboo. (line 12)

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**(2)**

(d)     DNA from wild pandas could also be obtained from blood samples. Suggest **two** advantages of using faeces, rather than blood samples, to obtain DNA from pandas.

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2 ...................................................................................................................

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**(2)**

**(Total 15 marks)**

**Q10.**          (a)     What is meant by a gene?

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**(2)**

The polymerase chain reaction (PCR) can be used to obtain many copies of a particular gene.

(b)     Explain how the strands of DNA are separated during the PCR.

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**(2)**

(c)     In a particular PCR, two different primers are added to the DNA.

(i)      Why are primers required?

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**(1)**

(ii)     Suggest why two different primers are required.

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**(1)**

(d)     Starting with a single molecule of DNA, the polymerase chain reaction was allowed to go through three complete cycles. How many molecules of DNA would be produced?

Answer .......................................

**(1)**

**(Total 7 marks)**

**Q11.**          There are wolves in many European countries. Scientists investigated the genetic diversity of these wolves. They collected samples of DNA from the mitochondria of wolves from different countries. For each sample they identified which haplotypes were present in the DNA. A haplotype is a particular sequence of bases on DNA. Mutations can produce new haplotypes.

|  |  |  |  |
| --- | --- | --- | --- |
|   | **Country** | **Number of wolvessampled** | **Number of differenthaplotypes inmitochondrial DNA** |
|   | Spain | 84 | 3 |
|   | Portugal | 19 | 2 |
|   | Italy | 101 | 1 |
|   | France | 7 | 1 |
|   | Bulgaria | 29 | 6 |
|   | Sweden | 93 | 1 |

The scientists wanted to find out whether one of the haplotypes in the Portuguese wolves was the same as one of those in the Spanish wolves. They used a restriction endonuclease, electrophoresis and a labelled DNA probe.

(a)     For what purpose did they use

(i)      the restriction endonuclease

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**(1)**

(ii)     electrophoresis?

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**(1)**

(b)     Explain why the labelled DNA probe could be used to find out whether the haplotypes were the same.

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**(2)**

(c)     The scientists analysed the DNA on the Y chromosome and the DNA in the mitochondria of the Swedish wolves. They concluded that the Swedish wolf population descended from one male wolf from Finland and one female wolf from Russia.

(i)      Explain why DNA on the Y chromosome helped them to reach this conclusion.

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**(1)**

(ii)     Suggest why DNA in the mitochondria helped them to reach this conclusion.

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**(1)**

Wolves eat different mammals. An ecologist investigated factors that affect wolf numbers in North America. He collected data from different field studies carried out in different places. The graph shows his results.



(d)     (i)      The wolf numbers are given per unit area. Explain why.

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**(2)**

(ii)     The ecologist calculated the total prey index for each of the places that had been studied. In order to do this, he gave each prey species a value based on how much food was available to wolves from the prey animal concerned. He called this value the prey index.

The ecologist considered that the prey index gave a better idea of the food available than the prey biomass in kg. Suggest why the prey index gives a better idea of food available.

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**(2)**

(e)      The ecologist calculated the total prey index by combining the prey indices and the total number of animals of each species present in 1000 km2. He plotted this information on the graph. What does the graph suggest about the factors that determine wolf numbers in North America? Explain your answer.

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**(2)**

**(Total 12 marks)**

**Q12.**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.

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**(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**

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

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**(2)**

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

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**[Extra space]** ................................................................................................

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**(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.

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**(2)**

(e)     Another group of scientists thought that pesticide resistance in some flies was related to increased activity of an enzyme called P450 monooxygenase (PM).
This enzyme breaks down insecticides.

The scientists obtained large numbers of resistant and non-resistant flies. They then set up the following experiments.

•        Non-resistant flies exposed to insecticide.

•        Resistant flies exposed to insecticide.

•        Resistant flies treated with an inhibitor of PM and then exposed to insecticide.

They then determined the percentage of flies that were dead at different times after being exposed to insecticide.

**Figure 2** shows their results.

**Figure 2**

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(i)      Explain why the scientists carried out the control experiment with the non-resistant flies.

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**(2)**

(ii)     The scientists concluded that the resistance of the flies to the insecticide is partly due to increased activity of PM but other factors are also involved.

Explain how these data support this conclusion.

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**[Extra space]** .......................................................................................

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**(4)**

**(Total 15 marks)**

**Q13.**          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.

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*(Extra space)* ........................................................................................

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**(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.

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**(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 ..................................................................................................

Explanation ...........................................................................................

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**(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.

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**(1)**

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

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**(1)**

**(Total 9 marks)**

**Q14.**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.

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**(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**.

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**(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**.

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*(Extra space)* ........................................................................................

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**(3)**

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

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**(2)**

**(Total 8 marks)**

**M1.**          (a)     only small amounts obtained / PCR increases the amount / mass of DNA;
so enough DNA available for genetic fingerprinting;

**2**

(b)     (i)      to separate the two strands of the DNA /
to break the hydrogen bonds;

*(Reject “unzip”)*

**1**

(ii)     short lengths / fragments of DNA / nucleotides /
single stranded DNA;

**1**

(iii)     to mark beginning and / or ends of the part of DNA needed /
for attachment of enzymes or nucleotides / initiator /
keeps strands apart;

**1**

(iv)    would not be denatured;
must be heated to 95 °C / must withstand high temps;

**2**

(c)     1 DNA extracted from sample;
2 DNA cut / hydrolysed into segments using restriction endonucleases;
3 must leave minisatellites / required core sequences intact;
4 DNA fragments separated using electrophoresis;
5 detail of process e.g. mixture put into wells on gel and electric
   current passed through;
6 immerse gel in alkaline solution / two strands of DNA separated;
7 Southern blotting / cover with nylon / absorbent paper (to absorb DNA);
8 DNA fixed to nylon / membrane using uv light
9 radioactive marker / probe added (which is picked up by required
   fragments) / complementary to minisatellites;
10 (areas with probe) identified using X-ray film / autoradiography;

**max 6**

(d)     adult 3;
this is only one which, (with number 1), can provide (all) the DNA
fragments which children have / all bars match;

*(Reject ‘genes’)*

**2**

**[15]**

**M2.**          (a)     to separate the two strands / break hydrogen bonds;

**1**

(b)     (i)      enables replication / sequencing to start (*allow keeps strands
separate*);

**1**

(ii)     joins DNA nucleotides (*not complementary bases*);

**1**

(c)     (i)      64;

**1**

(ii)     replication of DNA from crime scene / tissue sample /
for DNA sequencing / gene cloning;

**1**

(d)     (transcription uses) RNA polymerase;
RNA nucleotides / uracil;
one (template) strand / PCR both strands;
start / stop codons;
(*accept enzyme separates strands*)

**2 max**

**[7]**

**M3.**          (a)     (i)      to separate polynucleotide strands / form single strands;

**1**

(ii)     not denatured (at 95°C);

**1**

(iii)     for binding of primers / nucleotides (to DNA strands);

**1**

(b)     (i)      doubling (of DNA) each cycle;
but very low numbers to start with, so appears flat then
exponential growth;

**2**

(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**

**[7]**

**M4.**         1   DNA heated to 90 to 95°C;
2   strands separate;
3   cooled / to temperature below 70°C
4   primers bind;
5   nucleotides attach;
6   by complementary base pairing;
7   temperature 70 - 75°C;
8   DNA polymerase joins nucleotides together;
9   cycle repeated;

**6 max**

**[6]**

**M5.**          (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]**

**M6.**          (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]**

**M7.**          (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;

**2**

(c)     (i)      toothbrush gives small sample of DNA / need more DNA
for analysis;
PCR gives many copies;

**2**

(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

**2 max**

(d)     (i)      PCR / amplification needed;

**1**

(ii)     other DNA present; need to identify ‘required’ DNA from rest;

**2**

**[15]**

**M8.**          (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]**

**M9.**          (a)     1       DNA is cut;

2       Using restriction enzyme;

3       Use electrophoresis;

4       Separates according to length / mass;

5       Southern blotting / transfer to (nylon) membrane;

6       Make single-stranded;

7       Apply probe;

8       Radioactive / fluorescent;

9       Reference to tandem repeats / VNTRs / minisatellites;

10     Autoradiography / eq;
8 and 10 should be consistent

**max 6**

(b)     (i)      All bands in cub which don’t come from mother;

Must be in father’s DNA fingerprint;

*Principle that all bands in cub must come from mother and father = 1*

**2**

(ii)     Select pairs with dissimilar DNA fingerprints;

**1**

(c)     (i)      Cells (from panda) in faeces / gut cells / blood cells;

**1**

(ii)     To increase amount of DNA / only small amount present;

**1**

(iii)     DNA / primer has specific base-sequence;

Reference to specific / complementary base-pairing;

**2**

(d)     Taking samples from animals causes stress / injury to animal;

Difficult to find animals;

Pandas are dangerous / threat to human;

**max 2**

**[15]**

**M10.**          (a)     a length of DNA;
that codes for a single protein / polypeptide;

**2**

(b)     by heating;
to break the H-bonds (between complementary bases);

**2**

(c)     (i)      to allow the DNA polymerase to attach / start addition of
nucleotides / mark start and end of sequence to be
copied / prevents strands re-joining;

**1**

(ii)     because the sequences at the ends of the target sequence
are different / one is at the beginning and one at the end;

**1**

(d)     8;

*accept 7*

**1**

**[7]**

**M11.**          (a)     (i)      To cut the DNA;

*Reject breakdown, cutting out*

**1**

(ii)     To separate the (pieces of) DNA;

**1**

(b)     Complimentary base sequence / complementary DNA; binds to both (haplotypes);

Label would show up in both;

*Idea of complimentarity required*

**2**

(c)     (i)      Y chromosome inherited / comes from male parents / only found in males;

**1**

(ii)     Mitochondria in egg / female gamete / no mitochondria come from sperm / male gamete;

**1**

(d)     (i)      Allows comparison;

Different (sized) areas covered;

**2**

(ii)     Wolves do not eat all of prey animal / do not eat (large) bones / skin;

Inedible parts make up different proportions / wolf eats different proportions;

**2**

(e)      Limited by food / prey; as prey increases so do wolf numbers / positive correlation;

Large range so other factors involved;

**2**

**[12]**

**M12.**(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]**

**M13.**         (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*

**1**

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

*Accept: small fragment has run off gel / travelled further*

**1**

**[9]**

**M14.**(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]**

**E1.**          (a)     This yielded good answers backed by common sense.

(b)     Most candidates scored well in part (i). For part (ii) many gave answers which were more suited to part (iii), then failed to offer a relevant answer for (iii). Part (iv) was well done by many.

(c)     Many centres are clearly going into this topic with sufficient depth for their candidates to achieve full credit. The main errors were in confusing the order of events and the exact role of X rays; some discussed a mixture of electrolysis and chromatography and a very few based their answers on ink-based fingerprinting.

Adult 3 was correctly identified in most scripts but some found the reason hard to explain. A discussion of matching ‘genes’ was a common error.

**E2.**          Although this question produced a wide range of marks, many candidates were able to obtain at least two of the marks available.

(a)     Most candidates gained this mark by referring to separation of the two strands or to the breaking of hydrogen bonds. Vague references to ‘uncoiling’ or ‘unwinding’ were not credited.

(b)     Most candidates obtained the mark in part (i) by referring to the role of primers in enabling sequencing to start. However, in part (ii), considerably fewer candidates correctly described the role of DNA polymerase in joining DNA nucleotides together. Many candidates suggested that this enzyme forms the hydrogen bonds between complementary bases.

(c)     Part (i) and part (ii) caused few difficulties for the majority of candidates. In part (i), most candidates correctly calculated that 64 DNA molecules would have been produced after six complete cycles. In part (ii), the use of the polymerase chain reaction was often linked to the amplification of DNA for forensic purposes or for gene cloning.

(d)     This proved to be an effective discriminator with only the best candidates obtaining both marks. Most candidates failed to consider the processes involved and simply referred to the products of PCR and transcription. These answers were not credited. Better candidates often referred to transcription using RNA polymerase, uracil and one template strand.

**E3.**          (a)     This question was intended to allow most students to gain marks using their recall of PCR. In the event, few candidates obtained all three marks.

(i)      Many candidates obtained this mark by explaining that heating to 95°C causes separation of DNA molecules into separate polynucleotide strands. Credit was not given to simple statements restricted to ‘breaking’ or ‘splitting’ DNA. A surprising number thought that the heating was to provide the optimum temperature for enzymes.

(ii)     This was probably the best answered part of the question and many candidates correctly explained that the enzyme is not denatured at the high temperatures used in PCR.

(iii)     Quite a few candidates wrongly thought that this was the optimum temperature for the enzyme, or the temperature at which the enzyme could start to attach free nucleotides to the complementary bases on single strands. Many correctly identified this as the temperature at which primers or nucleotides can bind (form H bonds) to single-stranded DNA.

(b)     (i)      There were many good answers from candidates who understood the concept that the number of DNA molecules doubles with each cycle during PCR but that growth in numbers is slow at first, due to the small number of DNA molecules at the start. Some candidates spoilt their answer by suggesting that there were no DNA molecules at the start.

(ii)     Many candidates obtained one mark for suggesting that either primers or nucleotides were used up, or that the enzymes lost activity. Quite a few of these candidates were then unable to explain clearly how their suggestion slowed or stopped DNA replication. Some candidates misinterpreted the graph and saw it as one of rate of enzyme action against time. This led to incorrect suggestions revolving around the idea of the saturation of active sites of enzymes. Credit was given to candidates who suggested that large numbers of DNA molecules produce so many single strands that they re-bind to each other, rather than forming new complementary strands.

**E4.**         This question gave the candidates the opportunity to display their knowledge of factual material from the specification.

There were some very good answers with many candidates gaining full marks. The events of the cycle were written in the correct sequence and precise details of the temperature regime were well remembered by the majority of candidates. The role of DNA polmerase was often only alluded to, or incorrectly attributed to complementary base pairing. Weaker candidates described either the process of DNA replication in a cell or, less commonly, confused the process with protein synthesis.

**E5.**          This question proved to be very demanding for many candidates, although better candidates were able to score all, or almost all, of the available marks. In (a) (i), many candidates recognised that different species of shark have different characteristics, but mostly failed to link these characteristics to proteins or to genes. In (a) (ii), some candidates did realise that each primer had a specific base sequence which would bind to the shark DNA by complementary base-pairing, but weaker candidates simply re-stated the information in the stem. In (a) (iii), many candidates limited their answers to stating that the DNA fragment had to be a different length in each species so it could be identified. How this enabled identification was not stated. On the other hand, good candidates wrote succinctly about electrophoresis separating DNA by size, and explained how the different length fragments would move different distances up the gel. Part (b) was poorly done by most candidates. It was common to find descriptions of genetic fingerprinting here. A few very weak students confused the ‘rungs’ with base pairs in the DNA molecule. Part (c) was the most accessible part of the question. A large proportion of candidates clearly knew the polymerase chain reaction and gave excellent accounts, often gaining full marks. A minority of very weak candidates confused PCR and transcription.

**E6.**          **Unit 2**

(a)     Most had no trouble here in identifying endonuclease.

(b)     This was badly done since few mentioned or implied that DNA was made of base pairs and that these occupied the same distance along the backbone. Many made it obvious that they meant the distance from one chain to the other or went on to write about the distances in the electrophoresis gel.

(c)     Although the correct box was often located, the second from the right was almost as commonly given. Appropriate justification for the choice was rewarded in (ii) although many candidates suggested fragment 6 was the fifth rather than the second largest.

(d)     All figures were given here with 5 only rarely chosen, 6 being most common.

**Unit 3**

In part (a), restriction enzyme was correctly identified by almost all candidates. However, part (b) attracted very poor answers. Few commented that DNA is composed of base pairs, or that all base pairs have the same length. Many simply re-stated the question. In (c) (i), most candidates correctly identified the box, but a significant number identified the penultimate box. These candidates explained in (c)(ii) that the *longest* fragments moved furthest. Although they had the correct idea that DNA separates according to length, they had the relationship the wrong way round. In (d), there were few correct answers. Most gave 6.

**E7.**          (a)     Most candidates made a reasonable attempt at this part of the question with many scoring maximum marks. However many began their answers at the wrong point and gave details of PCR before describing genetic fingerprinting. The order of the process was also often confused, with many separating the strands of DNA before electrophoresis, or putting in a probe at the start of electrophoresis. Another misconception was the use of X-rays to identify the probe.

(b)     Most realised that cells would be removed from the mouth area when brushing the teeth and many then continued correctly to identify DNA as being in those cells. Few showed a better understanding by referring to the fact that the DNA was in the nucleus. The commonest misconception here was that saliva would contain DNA.

(c)     (i)      Many realised that PCR was being used to increase the amount of DNA but failed to link this to the low initial amount of DNA.

(ii)     This was a challenge for all but the best. The most common correct answer as to the difference was that PCR required heating to break the hydrogen bonds. Many candidates were of the opinion that PCR was conservative replication of DNA, while semi-conservative DNA replication occurred in cells.

(d)     (i)      Many realised that, with a small sample, it would take time to copy the genetic material.

(ii)     Only the better candidates appreciated that the sample would be contaminated with other DNA, and went on to recognise that it would take time to identify this or remove it. There were many vague answers about contaminants and the need to decontaminate.

**E8.**          (a)     Figure 2 showed that the male involved in this cross possessed two alleles and this should have alerted candidates to the fact that the gene concerned was not sex-linked. Most of those who identified it as being autosomal, were able to explain that the probability of the next child having thalassaemia was one in four. Rather less success was enjoyed, however, when it came to combining probabilities, and the mathematics of multiplying fractions was evidently beyond the ability of a number of candidates.

(b)     Part (i) was generally answered well by those candidates who distinguished between the processes of electrophoresis, chromatography and centrifugation. The basis of separation in this case is the difference in charge and, in DNA, this translates into differences in length. The numerous references to such features as solubility suggested that the distinction between these three processes was not always secure. The difficulties encountered by many in part (ii) stemmed largely from confusion between the terms allele, base and probe. There was, as a result, much inaccurate biology and little opportunity for awarding credit.

**E9.**          (a)     Some candidates could not isolate the essential information. Frequently, candidates included the detail about PCR in their responses before embarking on genetic fingerprinting. Maximum marks were regularly scored however.

(b)     Some candidates experienced problems explaining the situation in part (i). Too often they failed to include reference to the maternal DNA in their argument. Most based their argument on matching cub DNA to the paternal DNA, but this would simply show a close relationship. The question wanted candidates to focus on establishing paternity. Many candidates failed to state the obvious in (ii). They regularly indicated that the genetic fingerprinting would be used to find out which pandas were related, without saying that unrelated pandas would have  dissimilar bands.

(c)     In (i) a significant number indicated that bamboo DNA would be present, which of course would not be a source of panda DNA. Others suggested that protein, saliva or other body fluids would be a source of DNA. The role of PCR in (ii) was understood by the vast majority. However some did erroneously believe that PCR could be used to separate DNA, or to make the molecule longer. In (iii), all too often the primer was simply described as being specific without reference to the sequence of bases. Many were more familiar with the complementary nature of the primer. Some gave detailed accounts of the role of the primer, without explaining how it could bind to panda DNA but not bacterial or bamboo DNA.

(d)     This part proved challenging, even for the most able. Most candidates appreciated that it would be easier to collect faeces but could not offer a reason. ‘Cheaper’ ‘easier’ and ‘quicker’ were frequent answers, without any details to support the argument. Avoidance of distress to the panda was noted by many. However most were more concerned with the expense of collecting blood. They also discussed at length the fact that red blood cells would not provide any DNA, so they assumed that faeces would be a more concentrated source of DNA.

**E10.**          (a)     Examiners were very disappointed to read so many incorrect definitions of a gene. The idea that a gene is a ‘strand of DNA’ seems a common misconception. Some indicated that a gene was the entire DNA or that it was part of a chromosome. Few referred to it coding for a polypeptide but instead gave a vague reference to ‘characteristics’ or used examples, e.g., eye colour. A significant number incorrectly believed that the gene was a protein.

(b)     The concept of breaking bonds between the strands was well understood. However some failed to name the bonds correctly. Others incorrectly suggested that the bonds were broken by the action of enzymes (often restriction enzymes) rather than heat. Nevertheless, many candidates scored both marks here.

(c)     A significant proportion of the candidates were unsure of the role of primers, and a larger number could not suggest why two different primers are required.

(d)     Many candidates gave the correct answer of 8. Among the incorrect answers, the commonest were sixteen or six.

**E11.**          (a)     Most candidates had some understanding of the function of restriction endonuclease but were not always sure of its role in the investigation described. Thus, there were numerous references to the enzyme “cutting out” particular sections of DNA, these pieces ranging from haplotypes, to genes and even chromosomes. Most candidates correctly suggested that electrophoresis would be involved in separating the DNA fragments, although some were clearly of the opinion that it was the chains of DNA that were separated.

(b)     Candidates were generally able to describe the complementary base sequence present on the probe but seldom progressed to explain how it could be used to show that the haplotypes concerned were the same.

(c)     The majority of candidates linked the Y-chromosome to male inheritance in part (i) although a significant number suggested that the Y-chromosome was inherited from the female. Part (ii) was targeted at stronger candidates, but very few could suggest that mitochondria could only be passed to the offspring in the cytoplasm of the egg.

(d)     The responses to part (i) suggested that while many candidates were aware that giving the units per unit area enabled comparison, they were uncertain as to what was being compared. The most frequent suggestion was that it allowed wolves to be compared with prey numbers. Others wrote about the territorial behaviour of wolves or suggested that the mobility of the animals made counting over a larger area too difficult. In part (ii), better candidates appreciated that wolves ate only part of their prey and that the amount eaten differed with different species of prey.

(e)      Although the positive correlation between prey index and wolf numbers was usually recognised, few progressed to state that this suggested that food must be limiting population size. Unfortunately, the few who pointed out that other factors might possibly be involved rarely linked this conclusion to the spread of data on the graph.

**E12.**(a)     Over half of students managed to communicate the idea that the same restriction enzymes cut at the same place / recognition sequence on DNA. Only just over 10 percent considered the context of the question and went on to say that this would give fragments containing the same gene (R).

(b)     About half of students obtained both marks in this part and over 40 percent obtained 1. Most managed to convey the idea that there was binding between complementary base sequences on a primer and DNA from an allele. The better answers conveyed the idea of a specific base sequence for each primer.

(c)     Some very good and clear answers were seen to this part and a third of students obtained 3 marks. These students identified the genotypes of L, M and N and explained how they identified them on the basis of the sizes of primer attached to each and how far the bands moved. Quite a large number went on at length about the offspring as represented by M but failed to identify L and N. A few thought that there were two types of offspring because there were two bands for M; they clearly did not understand the simple genetics of the cross.

(d)     This proved far more challenging than expected. The examiners were looking for the idea that chromosomes would be visible (as separate structures) and thus the scientists could see to which chromosome the probe was attached. Fewer than 10 percent got both marks.

(e)     (i)      There were quite a large number of attempts at generic ‘How Science Works’ answers to this part. 50 percent of students obtained 1 mark, usually for suggesting that the control was to see the effect of the insecticide or that it was for comparison with resistant flies. However, only around 10 percent gave both of these.

(ii)     Most students focused on one aspect of the results and scored 2 marks. They frequently spotted that if the enzyme was the only factor in resistance, then the results for the controls and the resistant flies with the inhibitor would be the same. Others focused on evidence for the statement by comparing the resistant flies with resistant flies with the inhibitor added.

**E13.**         (a)      (i)      Although the negative correlation was usually indicated, only the better students appreciated the wide range in the age of onset or the overlap in values. Very few students were aware that the wide range in age of onset for the same number of CAG repeats suggests that other factors may also be involved.

(ii)      A minority of weaker students gave answers that were out of context. Some thought that the allele for Huntington’s disease is recessive and would therefore be passed on to offspring without a person knowing. Others thought that people with Huntington’s disease would survive well into adulthood and then reproduce.

(b)     (i)       Most students correctly identified Person **K** as testing positive for Huntington’s disease. They went on to explain that this person has the fragment that moved the shortest distance and linked this to a greater number of CAG repeats. A minority of students failed to link correctly the distance moved with the length of the fragment.

(ii)      Students who failed to gain credit often referred to using DNA sequencing or probes to highlight sequences and ‘restriction mapping’.

(iii)     Misconceptions seen in responses by weaker students included partial digestion of DNA, Person **L** only having one allele of this gene and the probe not being able to attach to the other fragment.

**E14.**(a)    About 80% of students identified the enzyme in this part as reverse transcriptase. The commonest wrong answer appeared to be restriction endonuclease.

(b)     Most students obtained one mark for stating that the DNA probe has a base sequence complementary to the DNA of allele A. A third obtained a second mark by going on to state that this allowed it to bind to the target DNA by base pairing, or that this meant only target DNA gives off green light.

(c)    (i)       About half of students obtained one mark in this part for noting that the more probe binding to allele A, the more green light there would be. Nearly a quarter obtained a second mark, usually for also noting that the light curve goes up exponentially (or described). Only a few, 14%, obtained all three marks. These students explained that this was because the amount of DNA doubles (approximately) with each PCR cycle.

(ii)     Answers to this part often made references to G being heterozygous because this person had *fewer* A alleles and thus *less* light was produced. The examiners were looking for more precise statements relating to half the amount of A with probe attached and half (approximately) the light produced (at any given time). A third of students obtained both marks.