19.1 Principles of Genetic Technology

Question Paper

Course	CIE A Level Biology
Section	19. Genetic Technology
Topic	19.1 Principles of Genetic Technology
Difficulty	Hard

Time allowed: 70

Score: /51

Percentage: /100

Question 1a

Spinocerebellar ataxia is a genetic condition that leads to a loss in the brain's ability to coordinate movement in hands, eyes, and speech. 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 spinocerebellar ataxia. People can be tested to determine the number of CAG repeats on this allele.

Fig.1 shows the age at which a sample of patients with spinocerebellar ataxia first developed symptoms and the number of CAG repeats in the allele causing spinocerebellar ataxia in each patient.

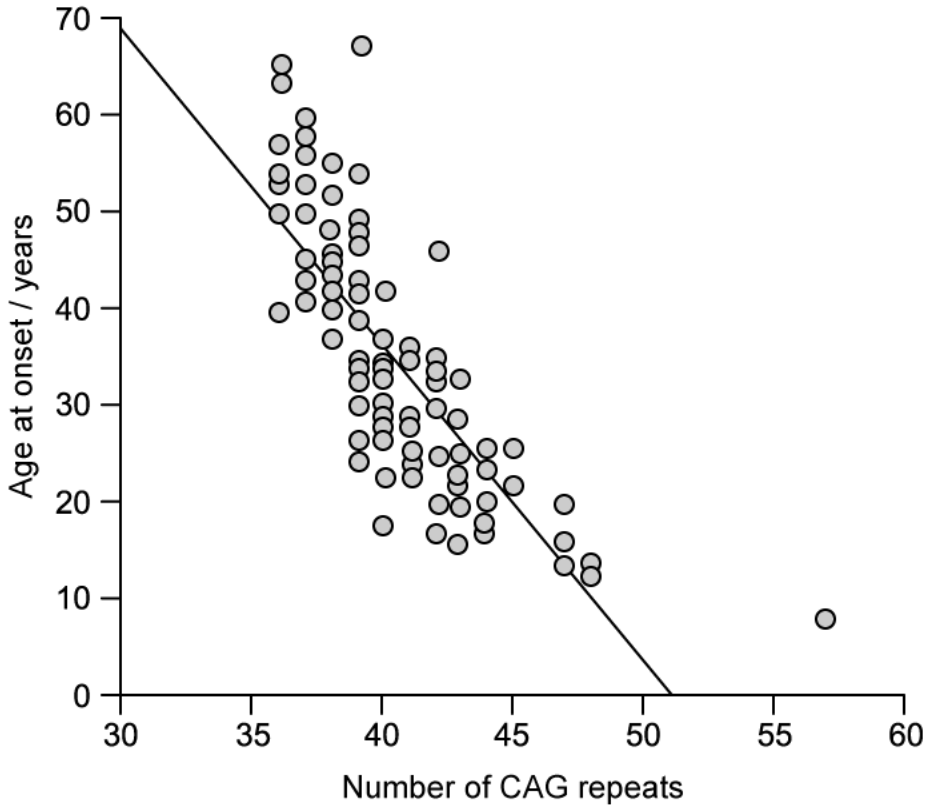

Fig. 1

A research assistant decided that the following conclusions can be drawn from Fig.1:

- More than 35 CAG repeats are needed for the development of spinocerebellar ataxia.
- It is possible to predict the age at which spinocerebellar ataxia will develop from the number of CAG repeats.

Use the information in Fig.1 to:

(i)
State how the research assistant concluded that more than 35 CAG repeats are needed for the development of spinocerebellar ataxia.

[1]

(ii)

Evaluate the conclusion that it is possible to predict the age at which spinocerebellar ataxia will develop from the number of CAG repeats.

[3]

[4 marks]

Question 1b

With reference to Fig.1, suggest why the allele that causes spinocerebellar ataxia is passed on in human populations despite the condition being fatal.

[1 mark]

Question 1c

DNA samples were taken from four people; **W**, **X**, **Y** and **Z**. The polymerase chain reaction (PCR) was used to produce many copies of the section of DNA containing CAG repeats obtained from each person. The DNA fragments were then separated by gel electrophoresis and detected using a radioactively labelled probe.

Fig. 2 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.

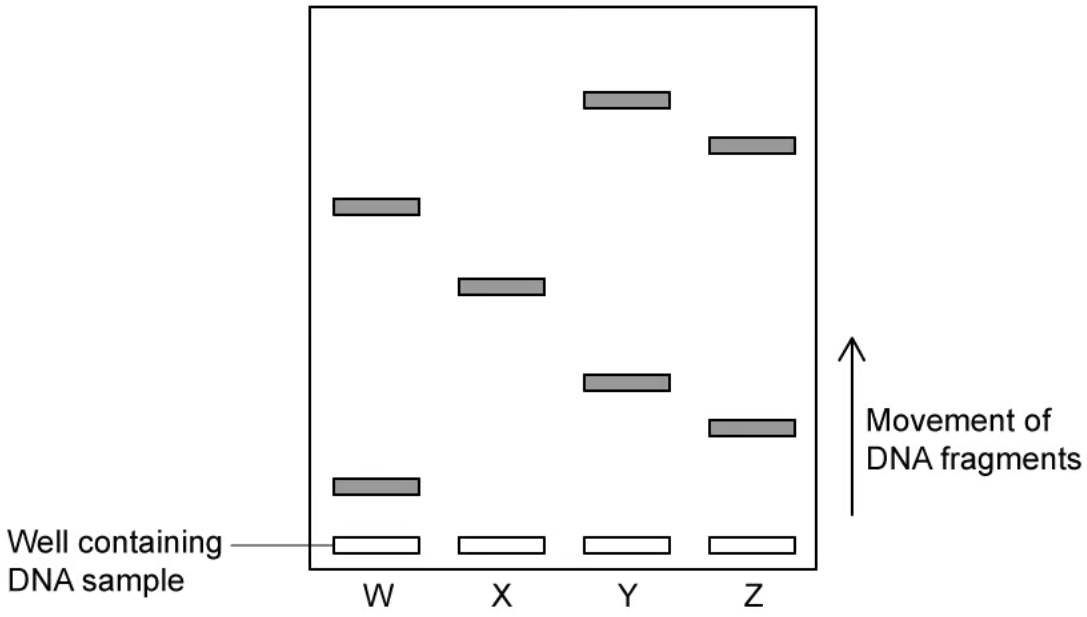

Fig. 2

Each individual usually has two bands.

Suggest why only one band was seen for person **X**.

[1 mark]

Question 1d

Only one of **W**, **X**, **Y** and **Z** tested positive for spinocerebellar ataxia.

(i)
Use Fig.1 to suggest which person tested positive for spinocerebellar ataxia.

[1]

(ii)
Explain your answer to part i).

[1]

[2 marks]

Question 2a

Leber's congenital amaurosis (LCA) is an autosomal recessive eye disease. LCA results in eye disorders, including severe loss of vision, at birth. LCA has been successfully treated by gene therapy, using a virus instead of a plasmid as the vector.

Adeno-associated virus (AAV) vectors containing the therapeutic allele were injected directly into the retina, the layer at the back of the eye containing the photoreceptor cells. People who had been blind from a young age were able to see again.

There is a risk associated with the injection method used to deliver the vectors, as it might cause the retina to detach, damaging vision. This method of delivery was first used for LCA before being trialled on other retinal diseases that gradually reduce the vision of people as they get older.

(i)

Suggest the main steps involved in creating recombinant DNA for this example of gene therapy.

[4]

(ii)

Explain why the fact that LCA is an autosomal recessive genetic disease makes it suitable for treatment with gene therapy.

[2]

(iii)

Suggest why the retinal injection method of gene therapy was used for LCA before it was trialled on other retinal diseases that gradually reduce the vision of people as they get older.

[2]

[8 marks]

Question 2b

Scientists tried to create an improved virus vector for gene therapy.

step 1	The scientists used a special form of the polymerase chain reaction (PCR). This form of PCR causes mutations in the DNA sequence of AAV by base substitution.
step 2	The viruses containing different base substitutions were tested. This was done by using the different viruses to deliver a new gene, the gene for green fluorescent protein (GFP), into the photoreceptor cells of mice, using the retinal injection method.
step 3	The best virus, known as 7m8, caused the photoreceptor cells in the retina of the mouse to fluoresce brightly, even when the recombinant virus was injected into the fluid inside the eye instead of into the retina itself.
step 4	The 7m8 virus was used to cure a mouse with LCA by injecting this virus containing the therapeutic allele into the fluid inside the eye of the mouse.

(i)

Suggest how errors occurring during PCR can cause base substitution mutations in the DNA sequence of AAV.

[3]

(ii)

Explain why the photoreceptor cells of the mouse fluoresced in step 3.

[2]

(iii)

Predict the impact of the 7m8 AAV on treatment for age-related retinal diseases.

[1]

[6 marks]

Question 3a

The interpupillary distance (IPD) is the distance in millimetres between the centres of the pupils of the eyes. Fig. 1 shows how IPD is measured.

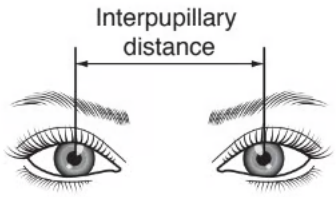

Fig. 1

IPD is one example of a characteristic of human facial structure that shows variation.

Fig. 2 shows the pattern of variation in IPD in a large sample of adults.

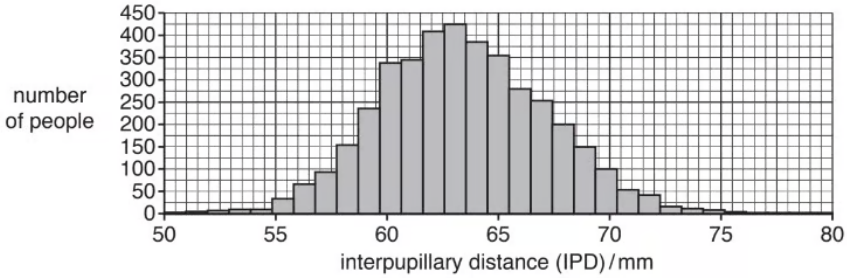

Fig. 2

(i)
Name the type of variation shown in Fig. 2.

[1]

(ii)
Suggest **and** explain how genes and the environment contribute to variation in IPD in humans.

[3]

[4 marks]

Question 3b

Individuals with an IPD of 70 mm or more have a mutation in the *PAX3* gene that results in less PAX3 protein being made.

The normal role of the PAX3 protein is to increase the expression of many other genes involved in embryonic development. These genes affect a range of phenotypic features such as facial structure, hearing and eye colour.

(i)

State the term that is used to describe a gene, such as *PAX3*, that controls the expression of other genes **and** suggest how the PAX3 protein controls the expression of other genes.

[3]

(ii)

Describe how microarray analysis could be used to identify the genes switched on by PAX3 in embryonic cells.

[5]

(iii)

The chimpanzee, *Pan troglodytes*, has DNA that is 98.5 % similar to humans, including possession of the *PAX3* gene. Investigations show that chimpanzees express higher levels of the PAX3 protein during embryonic development than humans.

Fig. 3 shows a chimpanzee, *Pan troglodytes*.

Fig. 3

Suggest how knowledge of the *PAX3* gene helps scientists explain how humans and chimpanzees are very different in facial structure, even though they have very similar DNA.

[3]

[11 marks]

Question 4a

Traditional techniques for genetically modifying organisms use three enzymes:

- Restriction endonuclease
- Reverse transcriptase
- DNA ligase

For example, these enzymes have been used to produce genetically modified (transgenic) pigs containing the *GFP* gene coding for green fluorescent protein, originally sourced from jellyfish.

Outline how these three enzymes could be used in genetically engineering a transgenic pig containing the *GFP* gene.

[3 marks]

Question 4b

A new technique that aims to cause a **deletion** in a gene uses an enzyme called Cas9 nuclease. It is injected into zygotes along with an RNA sequence (the guide RNA) that is complementary to a target gene. The Cas9 nuclease causes a deletion in the target gene in the zygotes, preventing the expression of that gene.

The toxicity and efficiency of the new technique was tested on four groups of pig zygotes. These pig zygotes were produced by IVF using:

- Ova from a female non-transgenic pig
- Sperm from a male transgenic pig whose somatic (body) cells contained one copy of the *GFP* gene per cell

The pig zygotes in three groups were injected with different concentrations of Cas9 nuclease and guide RNA **targeted at the *GFP* gene**.

The fourth group of pig zygotes (control group) was **not** injected with Cas9 nuclease and guide RNA.

Explain why the *GFP* gene was chosen for testing the new technique.

[2 marks]

Question 4c

Some of the zygotes in each group survived and after six days each had developed into a group of cells called a blastocyst.

The blastocysts were counted using a light microscope. A filter was then added to the microscope, so that only blastocysts expressing the green fluorescent protein showed up. These were counted and the results are summarised in Table 1.

Table 1

concentration of Cas9 nuclease and guide RNA / ng mm^{-3}	number of blastocysts seen under white light	number of blastocysts seen under filter
0 (control)	68	46
10	40	0
20	24	0
50	15	0

(i)
Calculate the percentage of zygotes in the control group that were transgenic.

Show your working.

[1]

(ii)

Explain whether the percentage you calculated for (**i**) is higher or lower than expected.

[1]

(iii)

Name a statistical test that would allow you to test the significance of the difference between the percentage you calculated in (**i**) and the expected percentage.

[1]

(iv)

State the best concentration of Cas9 nuclease and guide RNA to use to cause a deletion in the *GFP* gene **and** give reasons for your choice.

[3]

[6 marks]

Question 4d

Fig. 1 shows the results from a second trial of the new technique, analysed by electrophoresis.

- Lanes **1–4** show DNA from four pigs born after Cas9 nuclease was used to cause a deletion in a target gene coding for a cell surface protein.
- Lane **5** shows DNA from their surrogate mother.
- Lane **6** shows DNA from another normal pig for comparison.

The size of the DNA fragments is given in kilobase pairs (kbp) as shown in Fig. 1. 1kbp is 1000 base pairs of DNA.

The target gene measures 6kbp and codes for a cell surface protein that is essential for the disease virus PRRSV to infect cells in the pig's body.

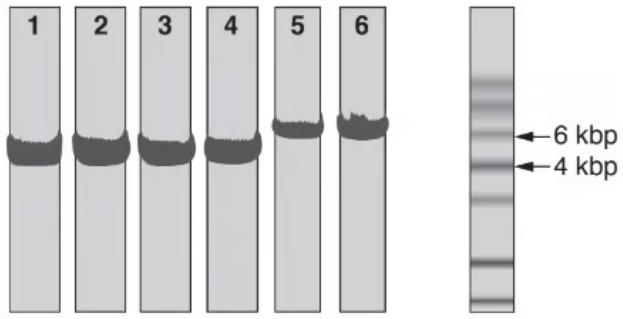

Fig. 1

Explain what Fig. 1 indicates about the success of the new technique in causing a deletion in a gene in pigs so that they show resistance to PRRSV.

[3 marks]