Model Answers: Hard

1a

a) i) The research assistant concluded that more than 35 CAG repeats are needed for the development of spinocerebellar ataxia because...

- There is no onset (at any age) when the number of repeats is less than (approximately) 35; [1 mark]

a) ii) Evaluative points regarding the conclusion that it is possible to predict the age at which spinocerebellar ataxia will develop from the number of CAG repeats could include...

In support of the conclusion

- There is a negative correlation between age of onset and number of CAG repeats **OR** the age of onset is lower when the individual has more repeats; [1 mark]
- The individual with the most CAG repeats (around 57 repeats) develops spinocerebellar ataxia at the lowest age; [1 mark]

Against the conclusion

A **maximum of two** of the following:

- Not all individuals fit the trend / negative correlation (identified above) **OR** some individuals with many CAG repeats develop spinocerebellar ataxia later than other individuals with fewer CAG repeats **OR** some individuals with few CAG repeats develop spinocerebellar ataxia earlier than other individuals with more CAG repeats; 1 mark]
- At any given number of repeats, spinocerebellar ataxia can develop at a range of ages **OR** at any given age of onset there are a range of ages at which spinocerebellar ataxia can develop; [1 mark]
- A correlation between two variables doesn't always mean that there is a causal link / that one variable has caused the other **OR** there may be variables other than the number of repeats that influences the age of onset; [1 mark]
- No statistical analysis has been provided to show the significance of the correlation; [1 mark]

[Total: 4 marks]

When evaluating a conclusion be sure to provide points both in support and against the conclusion for full marks.

1b

b) The allele is passed on in human populations because...

- Age of onset can be high / symptoms appear later in life/adulthood **SO** individuals have already had children / the allele/gene has been passed on (by the time they are aware they have the condition); [1 mark]

[Total: 1 mark]

As with Huntington's disease, spinocerebellar ataxia has a late onset, so it is possible for parents to pass the alleles to their children before being aware of the condition. Harmful genetic conditions with an early onset are more likely to affect an individual's ability to survive and pass on their alleles and so are more likely to be lost from the gene pool.

It is also possible that a recessive condition can remain in the gene pool due to the presence of carriers in a population, but as the question refers to Fig. 2.1 your answer points about recessive alleles will not be credited here.

1c

c) Only one band was seen for person **X** because…

Any **one** of the following:

- They are homozygous; [1 mark]
- (CAG) fragments in the alleles are the same length/size/mass; [1 mark]

[Total: 1 mark]

Each band is equivalent to a fragment of the same length/mass. Usually, individuals have a different number of CAG repeats on each allele and are therefore heterozygous. Person **X** has two alleles that have the same number of repeats and are therefore the same size. Fragments of the same size travel the same distance through the gel and will show up as a single band.

1d

d) i) The person who tested positive for spinocerebellar ataxia was…

- Person **W**; [1 mark]

d) ii) We can say this because…

- **W** has the band that travelled the shortest distance **SO** has the largest (DNA) fragment / number of CAG repeats; [1 mark]

[Total: 2 marks]

You must relate the distance travelled to the size of the DNA fragment. Smaller fragments suffer less resistance and therefore travel further through the gel. Spinocerebellar ataxia patients have many CAG repeats which therefore results in longer fragments (more repeats means more nucleotides which means longer nucleotide chains), therefore you should expect the patient's band to move the least.

2a

a) i) The main steps involved in creating recombinant DNA for this example of gene therapy are…

Any **four** of the following:

- Synthesise/obtain the therapeutic/correct allele / produce (c)DNA for the correct allele; [1 mark]
- From the mRNA of a healthy person / from a gene library; [1 mark]
- Use a probe / carry out electrophoresis/DNA sequencing to identify the therapeutic/correct allele; [1 mark]
- Use PCR to amplify allele/gene/DNA; [1 mark]
- A restriction enzyme/endonuclease is used **AND** its role is to cut DNA at a specific recognition site/sequence (to isolate the required gene); [1 mark]
- (DNA) ligase is used **AND** its role is to join together DNA nucleotides / catalyse the formation of phosphodiester bonds (in the DNA backbone); [1 mark]
- Add a promoter region; [1 mark]

a) ii) The fact that LCA is an autosomal recessive genetic disease makes it suitable for treatment with gene therapy because…

Any **two** of the following:

- (It is possible to) insert the correct DNA/allele; [1 mark]
- (Only) one allele/copy is needed per cell; [1 mark]
- To cure disease / restore vision; [1 mark]

- (As this allows the cells of the eye) to synthesise functional protein; [1 mark]
- (There is) no need to edit/remove the faulty allele (as would be the case if faulty allele was dominant); [1 mark]

a) iii) 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 because...

Any **two** of the following:

- LCA patients are already blind / have lost vision; [1 mark]
- It is (therefore) less likely to harm/worsen vision for LCA patients; [1 mark]
- Other age-related diseases may involve many genes / dominant alleles; [1 mark]

[Total: 8 marks]

i) You need to make sure your answer aligns with the **specific example** given in the question, so don't just regurgitate your knowledge of genetic engineering without altering your answers to suit the information provided. For example the question tells you that a **viral vector** has been used so any references to plasmids would be incorrect, and references to genes being located in pancreas cells would also be incorrect (this question is about an eye disease and not about producing insulin!).

Note that the terms gene and allele should not be used interchangeably; here the gene codes for a protein and it is the **allele** of the gene that determines whether or not the protein functions correctly.

2b

b) i) Errors occurring during PCR can cause base substitution mutations in the DNA sequence of AAV because...

Any **three** of the following:

- Wrong base added / wrong bases pair up...; [1 mark]
- ...To template DNA/strand; [1 mark]
- *Taq* polymerase is inaccurate / no proofreading occurs; [1 mark]
- In extension/elongation stage; [1 mark]
- Mistake/mutation is replicated/copied (many times); [1 mark]
- High temperature speeds up replication and increases the chance of mistakes; [1 mark]

b) ii) The photoreceptor cells of the mouse fluoresced in step 3 because...

Any **two** of the following:

- AAV/7m8/virus crossed the fluid / reached the photoreceptors/retina; [1 mark]
- (The virus) delivered (new/GFP) allele/DNA to photoreceptors/retina cells; [1 mark]
- (GFP) gene/DNA expressed in photoreceptors/retina cells; [1 mark]
- GFP/protein product is fluorescent; [1 mark]

b) iii) A prediction of the impact of the 7m8 AAV on treatment for age-related retinal diseases is...

- (7m8) will decrease the risk involved with / increase treatment by gene therapy; [1 mark]

[Total: 6 marks]

ii) Don't confuse the gene for GFP, which is a section of DNA, with the protein product GFP, which glows green under UV light.

3a

a)

i) The type of variation shown in Fig. 2 is…

- Continuous (variation); [1 mark]

ii) Genes and the environment contribute to variation in IPD in humans as follows…

Any **three** of the following:

- Several/many genes; [1 mark]
- (Genes have an) additive effect / (gene products) interact; [1 mark]
- The environment has a large/significant effect (on IPD); [1 mark]
- (Named) environmental factors affect gene expression (e.g. diet, stress); [1 mark]
- Age / diet / disease / parasites / chemicals (e.g. in the uterus during development) can affect the phenotype; [1 mark]

Accept *mutagens or alcohol for chemicals in marking point 5.*

Accept *development or IPD for phenotype in marking point 5.*

[Total: 4 marks]

It can be difficult to explain the genetic and environmental factors that influence specific types of variation.

Continuous variation in a particular characteristic, such as IPD, occurs as the result of multiple genes interacting with each other; such genes can be referred to as polygenes. These genes can have an additive effect, each having a small effect on the overall phenotype of an organism.

The environment also has an impact on such characteristics. In this instance factors such as age, diet, and exposure to chemicals during development may all influence IPD. For example, overall growth of the skull may be affected by environmental factors such that the orbits (eye sockets) develop closer together or further apart, which will also affect the interpupillary distance.

3b

b) i) The term that is used to describe a gene, such as *PAX3*, that controls the expression of other genes is…

- Regulator(y) (gene); [1 mark]

AND

The PAX3 protein controls the expression of other genes by…

Any **two** of the following:

- The gene codes for (a protein that is) a transcription factor / activator/inducer; [1 mark]
- (The protein expressed from the gene) binds to DNA; [1 mark]
- The DNA binding site is the) promoter/enhancer (region); [1 mark]
- (Binding to the promoter) enables RNA polymerase (to bind to the start of a gene); [1 mark]

b) ii) Microarray analysis could be used to identify the genes switched on by PAX3 in embryonic cells as follows…

Any **five** of the following:

- Take mRNA from (embryonic) cell (that is expressing *PAX3*); [1 mark]
- Make cDNA from the mRNA; [1 mark]
- Add a fluorescent tag to the (c)DNA (that has just been made from the mRNA); [1

mark]

- The microarray has DNA probes for known genes; [1 mark]
- cDNA hybridises with / binds to complementary base pairs (on the relevant probes); [1 mark]
- Fluorescence indicates expression (of genes); [1 mark]
- Wash off excess cDNA after hybridisation **OR** use UV to see fluorescence **OR** intensity of the fluorescence gives a quantitative measure (of expression); [1 mark]

b) iii) 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 because...

Any **three** of the following:

- Small genetic differences / difference in a single gene can have a large phenotype effect; [1 mark]
- (This is) because *PAX3* regulates many other genes; [1 mark]
- There is a different pattern of gene expression / different genes are expressed (in chimps compared to humans); [1 mark]
- More PAX3 (protein) in chimps reduces chimp IPD / distance between chimp eyes / face width; [1 mark]

[Total: 11 marks]

i) Read the question carefully here; you are asked to name the type of **gene** and not the type of protein expressed from the gene.

ii) Make sure that you are familiar with the details of microarray analysis. For example you should know that it is **mRNA** that is extracted from the cell of interest and not DNA; this is because the mRNA present will tell scientists which genes are being expressed and not just which genes are present in the cell. **cDNA** can then be made from this mRNA and labelled with **fluorescent** tags before being allowed to bind to complementary DNA probes that are **already present** on the microarray. Washing the microarray removes any unbound pieces of cDNA and then **UV light** can be used to reveal whether or not the cell is expressing particular genes.

iii) This is a 'suggest' question, meaning that you are expected to apply knowledge of gene function to this unfamiliar scenario. The fact that PAX3 protein has a regulatory role that affects the expression of 'many other genes' (see question stem) is key here. If PAX3 protein is expressed differently in chimps then this could lead to different expression of multiple other genes in the cells of developing chimps. The image provided allows you to be specific about the effects of different PAX3 levels on facial structure.

4a

a) These three enzymes could be used in genetically engineering a transgenic pig containing the *GFP* gene by...

- Restriction endonuclease/enzyme cuts vector/plasmid; [1 mark]
- Reverse transcriptase to make cDNA using mRNA; [1 mark]
- DNA ligase joins sugar phosphate backbone (between gene and vector) **OR** DNA ligase forms phosphodiester bonds (between gene and vector); [1 mark]

[Total: 3 marks]

It is very important to include enough detail in your answers, for example saying that ligase

joins the DNA together is not the same as saying that it catalyses the formation of the phosphodiester bonds in the sugar phosphate backbone of the DNA. Think about whether your answer contains enough A Level detail as opposed to something you remember from your GCSE level studies.

4b

b) The *GFP* gene was chosen for testing the new technique because...

- Marker; [1 mark]
- No fluorescence means GFP gene was deleted; [1 mark]

[Total: 2 marks]

A marker gene is a gene used to help scientists identify whether genetic modification has been successful. GFP is a good gene to use because you can see visibly if the gene is present or not, rather than having to do complex DNA sequencing to identify the DNA of a genetically engineered organism.

4c

c) i) The percentage of zygotes in the control group that were transgenic is...

- 67.6 or 68%; [1 mark]

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

% of transgenic zygotes = $\frac{46}{68} \times 100 = 67.6\%$ [1 mark]

c) ii) The percentage is...

- Higher, as expect 50% (of offspring to get GFP gene from heterozygous male); [1 mark]

Accept: lower, as expect 50%, (if answer to (c)(i) less than 50%); [1 mark]

c) iii) A statistical test that would allow you to test the significance of the difference between the percentage you calculated and the expected percentage is...

- χ^2 / chi-squared; [1 mark]

c) iv) The best concentration of Cas9 nuclease and guide RNA to use to cause a deletion in the *GFP* gene is...

- 10 ng mm^{-3}; [1 mark]

Plus any **two** of the following:

- More blastocysts; [1 mark]
- Less toxic; [1 mark]
- No blastocysts seen under filter / as successful as higher concentrations / all blastocysts have deleted GFP; [1 mark]

[Total: 6 marks]

Make sure you include the units in your answer.

4d

d) Fig. 1 indicates that...

Any **three** of the following:

- Lanes **1–4** show 4 kbp fragment; [1 mark]
- So technique is 100% successful; [1 mark]
- (6 kbp gene has) 2 kbp deleted/lost; [1 mark]
- Pigs (**1–4**) have no (normal cell surface) protein; [1 mark]
- PRRSV/virus cannot infect the cells/pigs (**1–4**); [1 mark]

[Total: 3 marks]