Q1.

A scientist produced transgenic zebrafish.

She obtained a gene from silverside fish. The gene codes for a growth hormone (GH).

She inserted copies of this *GH* gene into plasmids. She then microinjected these recombinant plasmids into fertilised egg cells of zebrafish.

(a) Describe how enzymes could be used to insert the *GH* gene into a plasmid.

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(2)

(b) Microinjection of DNA into fertilised egg cells is a frequent method of producing transgenic fish. However, the insertion of the transferred gene into nuclear DNA may be delayed. Consequently, the offspring of transgenic fish may not possess the desired characteristic.

Suggest and explain how delayed insertion of the *GH* gene could produce offspring of transgenic fish without the desired characteristic.

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(2)

The scientist investigated whether the transferred *GH* gene increased the growth of transgenic zebrafish. She microinjected 2000 fertilised egg cells with the *GH* plasmid and left 2000 fertilised egg cells untreated. After 12 months, she determined the mean mass of the transgenic and non-transgenic fish.

The results the scientist obtained are shown in the table below.

A value of ±2 × SD from the mean includes over 95% of the data.

Type of zebrafish	**Mean mass of zebrafish / g (± 2 × SD)**
Transgenic	1.79 (± 0.37)
Non-transgenic	0.68 (± 0.13)

(c) Using the table above, what can you conclude about the effectiveness of the *GH* gene on the growth of zebrafish?

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(2)

(d) Explain how **two** features of the design of this investigation helped to ensure the validity of any conclusions obtained.

Do **not** include calculating the mean or SD in your answer.

1 _____

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2 _____

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(2)

(Total 8 marks)

Q2.

Soybeans are used in a number of processed foods. However, soybeans contain a protein known as P34 that causes an allergic response in some people. Scientists have created transgenic soybeans that produce single-stranded cDNA, which prevents transcription of the *P34* gene. They used recombinant plasmids as vectors to transform soybean cells. After they had screened these cells for production of the P34 protein, they cultured the transformed cells to form soybean plants.

(d) Suggest how single-stranded cDNA could prevent transcription of the *P34* gene.

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(1)

(e) Describe the roles of **two** named types of enzymes used to insert DNA fragments into plasmids.

Type of enzyme _____

Role _____

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Type of enzyme _____

Role _____

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(2)

(f) The soybean cells were screened for the presence of the P34 protein. This process involved the use of gel electrophoresis to separate proteins extracted from soybean cells.

Suggest **two** features of the structure of different proteins that enable them to be separated by gel electrophoresis.

1. _____

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2. _____

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(2)

Q3.

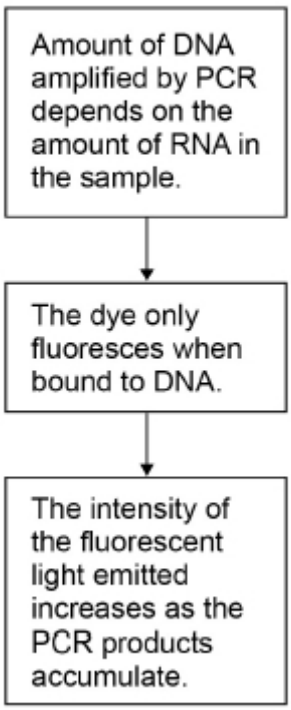
One way to detect and measure accurately the amount of RNA in a tissue sample is by RT-PCR (reverse transcriptase-polymerase chain reaction).

RT-PCR uses a reaction mixture containing:

- the sample for testing
- reverse transcriptase
- DNA nucleotides
- primers
- DNA polymerase
- fluorescent dye.

The principle behind this method is shown in **Figure 1**.

Figure 1



(a) Explain the role of reverse transcriptase in RT-PCR.

(1)

(b) Explain the role of DNA polymerase in RT-PCR.

(1)

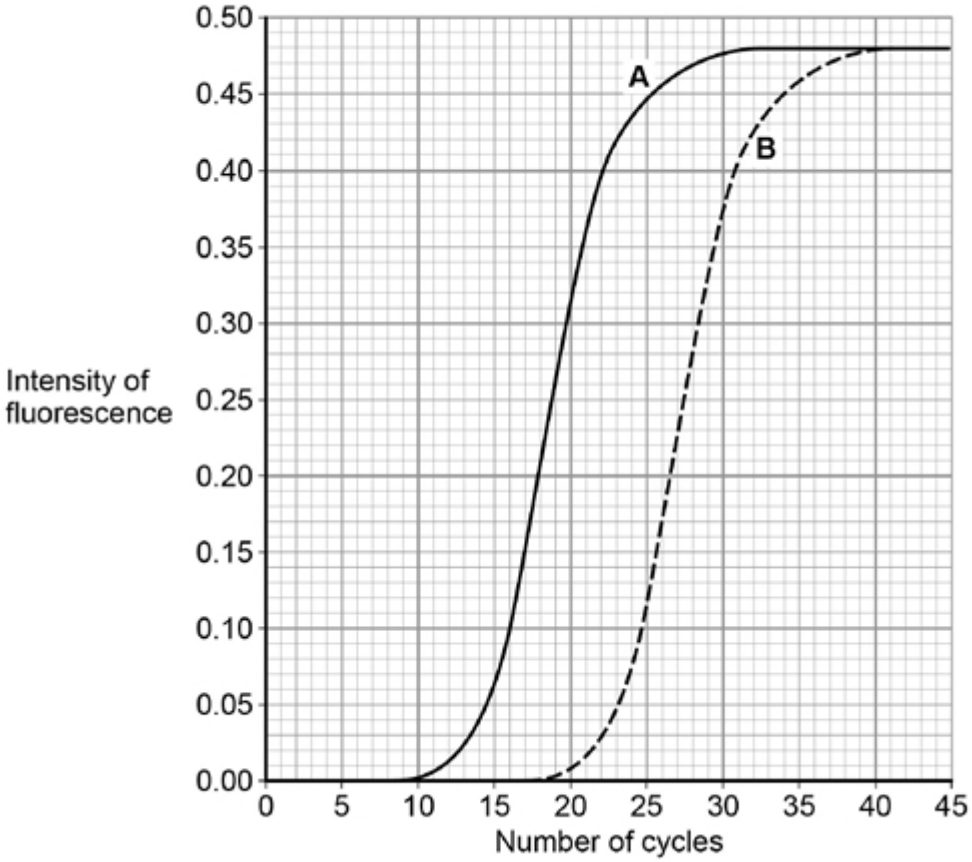
(c) Any DNA in the sample is hydrolysed by enzymes before the sample is added to the reaction mixture.

Explain why.

(2)

(d) **Figure 2** shows the results from using RT-PCR to detect RNA in two different samples, **A** and **B**.

Figure 2



A quantitative comparison can be made of the amount of RNA in samples **A** and **B**. This involves determining the number of cycles required to reach 50% maximum concentration of DNA (**C**).

The amount of RNA in a sample can be measured as: $\frac{1}{\mathbf{C}}$

Use this information to calculate the ratio for RNA content in sample **A** : RNA content in sample **B**.

Answer _____

(2)

(e) Suggest **one** reason why DNA replication stops in the polymerase chain reaction.

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(1)

(f) Scientists have used the RT-PCR method to detect the presence of different RNA viruses in patients suffering from respiratory diseases.

The scientists produced a variety of primers for this procedure.

Explain why.

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(2)

(Total 9 marks)

Q4.

People suffering from pituitary dwarfism do not make enough human growth hormone (HGH). They can be treated using injections of HGH.

A geneticist wants to transform the bacterium, *Escherichia coli*, to make HGH by adding the gene coding for HGH.

The geneticist could obtain the *HGH* gene using any one of three methods.

1. Use restriction enzymes to cut out a fragment of DNA containing the *HGH* gene from a human genome.
2. Convert mRNA for HGH into cDNA using reverse transcriptase.
3. Create the *HGH* gene using a 'gene machine'.

(a) The geneticist decided **not** to use restriction enzymes to cut out a fragment of DNA containing the *HGH* gene from a human genome. She made this decision because only methods 2 and 3 would produce DNA that *E. coli* could use to make HGH.

Explain why only methods 2 and 3 would produce DNA that *E. coli* could use to make HGH.

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(2)

(b) The geneticist concluded it would be faster to create the *HGH* gene using a gene machine than by using reverse transcriptase to convert mRNA for HGH into cDNA.

Suggest why the geneticist reached this conclusion.

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(1)

(c) After obtaining copies of the *HGH* gene, the geneticist will attempt to insert them into plasmid vectors.

Describe how the geneticist would attempt to insert copies of the *HGH* gene into these plasmids.

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(3)

(d) The geneticist plans to use the plasmids containing the *HGH* gene to try to transform cells of *E. coli*. She knows that some *E. coli* might not take up the plasmid.

To enable her to identify which bacteria have taken up the plasmid with the *HGH* gene, the plasmids she intends to use contain a gene that codes for a green fluorescent protein (GFP). Bacteria that contain this plasmid glow green under UV light.

Suggest **one** advantage of using this gene for GFP to identify bacteria that have taken up plasmids.

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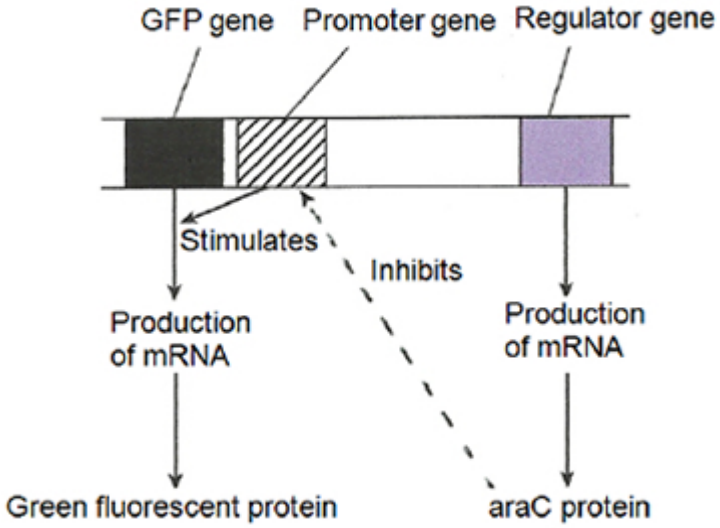
(1)

The diagram below shows part of the plasmid containing the gene that codes for GFP. It also shows the roles of two genes that control the GFP gene.



(e) Arabinose is a sugar that can bind to the araC protein.

Use information in the diagram to suggest why the geneticist must include arabinose in the agar on which she hopes to grow *E. coli* containing the transgenic plasmids.

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(2)

(Total 9 marks)

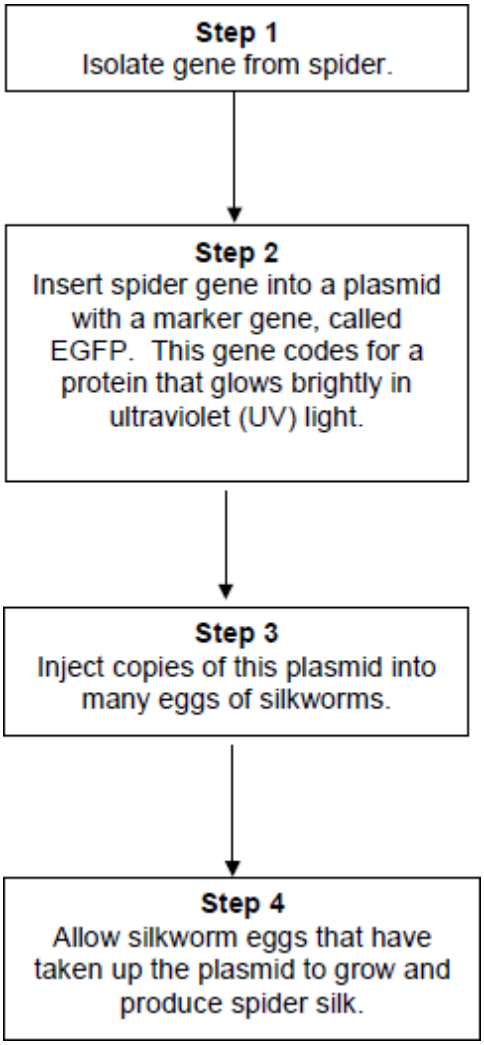
Q5.

Silkworms secrete silk fibres, which are harvested and used to manufacture silk fabric.

Scientists have produced genetically modified (GM) silkworms that contain a gene from a spider.

The GM silkworms secrete fibres made of spider web protein (spider silk), which is stronger than normal silk fibre protein.

The method the scientists used is shown in the figure below.



(a) Suggest why the plasmids were injected into the eggs of silkworms, rather than into the silkworms.

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(2)

(b) Suggest why the scientists used a marker gene and why they used the EGFP gene.

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(2)

The scientists ensured the spider gene was expressed only in cells within the silk glands.

(c) What would the scientists have inserted into the plasmid along with the spider gene to ensure that the spider gene was only expressed in the silk glands of the silkworms?

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(1)

(d) Suggest **two** reasons why it was important that the spider gene was expressed only in the silk glands of the silkworms.

1. _____

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2. _____

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(2)

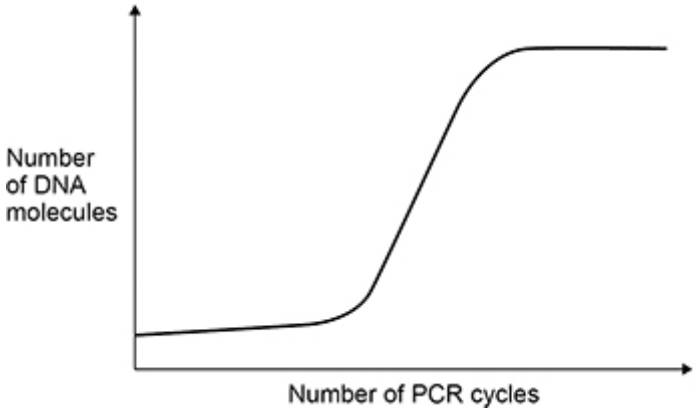
(Total 7 marks)

Q6.

(a) Describe and explain how the polymerase chain reaction (PCR) is used to amplify a DNA fragment.

(4)

The figure below shows the number of DNA molecules produced using a PCR.



(b) Explain the shape of the curve in the figure above.

(2)

(Total 6 marks)