Manipulating Genomes

1. Which statement correctly describes a difference between somatic and germ line gene therapy?

A. Germ line therapy involves the use of liposomes; somatic therapy involves use of viral vectors.
B. Somatic therapy can target specific tissues in need of treatment, germ line therapy cannot.
C. Somatic therapy is most successful when targeting single gene defects, but germ line therapy can target multiple defects.
D. Long term success is theoretically more likely with somatic cell therapy than germ line therapy.

Your answer ☐

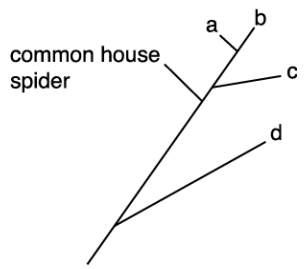
[1]

2. Scientists sequenced the DNA of the common house spider and four other species a, b, c and d that look similar. Analysis revealed the following differences from the DNA of the common house spider.

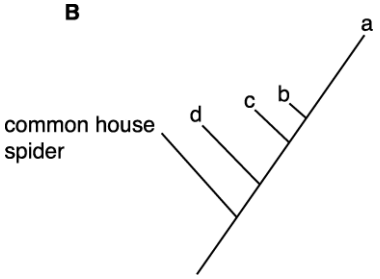
Species	Number of differences from DNA of common house spider
a	23
b	72
c	6
d	18

Which phylogenetic tree matches these data?

A



B



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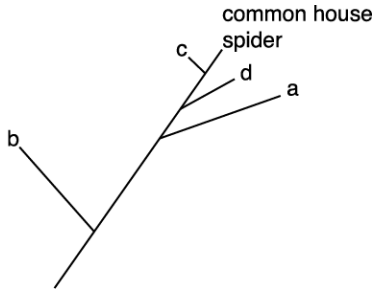
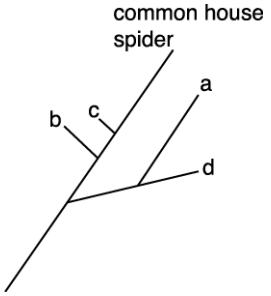


D



Your answer ☐

[1]

3. What is the main advantage of the polymerase chain reaction (PCR) when it is used as part of the process to sequence the genome of an endangered species?

A it is cheaper than rearing animals
B it never makes mistakes
C it reproduces DNA rapidly
D only a small sample of DNA is required

Your answer ☐

[1]

4. Which of the following statements about gene therapy is **not** correct?

A changes resulting from gene therapy cannot be passed on to offspring
B germ-line gene therapy affects the whole organism
C gene therapy is a form of genetic engineering
D somatic cell gene therapy can only affect a limited number of cells

Your answer ☐

[1]

5. Which of the following is **not** a valid concern about the use of genetic modification?

A that antibiotic resistance genes could transfer to pathogenic bacteria
B that herbicide resistance genes could be transferred to wild species
C that certain seeds might not be available for use by poor farmers
D that the use of human embryos in stem cell production is unethical

Your answer ☐

[1]

6. Gene sequencing has a number of uses.

Which of the following is **not** a use of gene sequencing?

A determining the amino acid sequence of a polypeptide
B the classification of newly-discovered organisms
C the polymerase chain reaction
D the selection of the correct vaccine in a disease outbreak

Your answer ☐

[1]

7. DNA fragments can be separated using gel electrophoresis.

Which of the following explains how gel electrophoresis is able to separate DNA fragments?

A DNA carries a negative charge and large fragments are pulled more strongly than small fragments towards the positive electrode.

B DNA carries a negative charge and small fragments are able to travel more quickly than large fragments towards the positive electrode.

C DNA carries a positive charge and large fragments are pulled more strongly than small fragments towards the negative electrode.

D DNA carries a positive charge and small fragments are able to travel more quickly than large fragments towards the negative electrode.

Your answer ☐

[1]

8. Which of the following substances is **not** required in DNA sequencing?

A DNA polymerase

B primers

C RNA nucleotides

D terminator bases

Your answer ☐

[1]

9. Lupus is an autoimmune disease that affects the skin, joints and internal organs.

Which of the following is likely to be an effective treatment for lupus?

A immunosuppressant drugs

B injection of antibodies from someone who does not suffer from lupus

C somatic gene therapy

D vaccination

Your answer ☐

[1]

10(a). In order to sequence the whole genome of an organism it may be necessary to sequence billions of nucleotides. The human genome is approximately 3.2 billion nucleotides long.

Sequencing DNA requires a series of steps.

Place the following steps in the correct sequence. The first and last ones have been done for you.

A. place sections in order by matching overlapping regions
B. cut DNA into sections of varying length
C. sequence short sections of DNA
D. amplify the DNA (create many copies)
E. extract samples of DNA from cells

E .... **A**

[2]

The development of high-throughput sequencing techniques has enabled whole genomes to be sequenced more rapidly. Table 17.1 compares a number of DNA sequencing techniques.

Technique	Rate of sequencing (Mb day^{-1})	Maximum length of nucleotide chain sequenced	Typical number of errors per 100 000 nucleotides
Sanger (chain termination technique)	6	1000	5
Roche pyrosequencing	750	500	50
SOLiD	5000	50	500
Helicos	5000	32	1000

Table 17.1

(b). The protein coded for in a gene is 200 amino acids in length. How many errors could be expected in the exons of the sequenced gene when using the least accurate sequencing technique shown in Table 17.1.

Answer..... **[2]**

(c). Roche pyrosequencing relies on building a chain of nucleotides against a template. It involves the following steps:

- Nucleotides are washed over the template in a specific order.
- When the correct nucleotide is present it joins the new chain.
- The addition of a nucleotide to the chain releases energy.
- The energy is used to activate a protein called luciferin.
- Light released by luciferin is detected.
- If two identical nucleotides are added together then the intensity of the light emitted is doubled.

Fig. 17.1 shows a readout from a pyrosequencing machine.

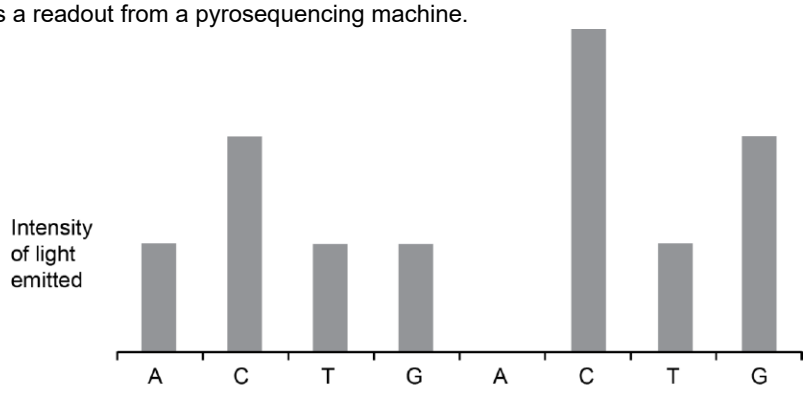


Fig. 17.1

Read off the sequence of bases in the length of DNA.

_____ **[1]**

11(a). Fred Sanger developed an effective DNA sequencing technique in 1977.

Define the term *DNA sequencing*.

_____

_____

_____ **[1]**

(b). The speed at which DNA can be sequenced has been increasing rapidly since the introduction of DNA sequencing.

The length of DNA that can be sequenced in a given time is measured in base pairs or kilobase pairs.

In 1980, the speed at which DNA could be sequenced by a single machine was approximately 500 **base pairs** per hour. In 2016 that speed had increased to approximately 50 million **kilobase pairs** per hour.

Calculate how many times faster the speed of DNA sequencing is in 2016 compared with 1980.

Answer ..... times faster **[2]**

(c). One technique that has allowed the speed of DNA sequencing to increase has been the development of nanopores.

Fig. 21 shows how nanopores can be used to sequence DNA.

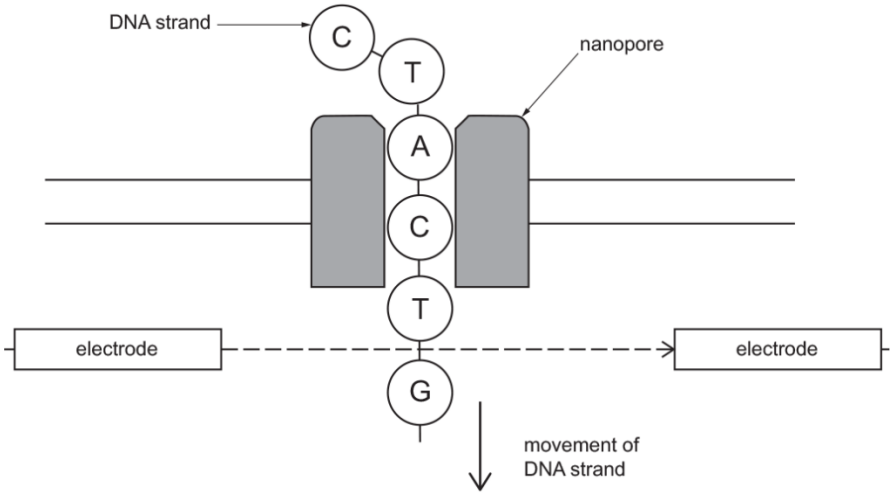


Fig. 21

i. State one development, other than nanopore technology, that has led to an increase in the speed at which DNA can be sequenced.

.....

.....

[1]

ii. Part of Fig. 21 is labelled **G**.

Use the table below to identify two differences between the part labelled **G** and the structure of a molecule of ATP.

	G	**Molecule of ATP**
Difference 1	....	....
Difference 2	....	....

[2]

iii. Explain how DNA sequencing allows the sequence of amino acids in a polypeptide to be predicted.

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.....

.....

[2]

(d). DNA sequencing can be used to determine the genome of an entire organism.

The first organism to have its entire genome sequenced was a virus.

Ebola is a virus that caused the death of over 11 000 people in West Africa between 2014 and 2016. The DNA of ebola virus has a rapid rate of mutation.

Since the first outbreak in 2014 scientists have been working to develop an effective vaccination against ebola.

Other scientists have developed a portable nanopore sequencing technique that could be used to sequence rapidly the entire ebola genome.

Outline how DNA sequencing and bioinformatics could be used to increase the effectiveness of a vaccination programme against ebola.

sequencing

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bioinformatics

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[4]

12.

i. A portion of a gene was sequenced from two members of the same family suspected of having a genetic disease.

The sequences are shown below:

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ACGGTATTGCTACTTGAATTACGT
ACGGTATTGAGCCTTGAATTACGT
```

What proportion of the sequence is different?

Answer = ... **[2]**

ii. To identify an allele that causes a genetic disease it must be sequenced accurately so that differences from the healthy allele are clear.

Using the information in **Table 17.1** decide which technique is best to use when sequencing a human gene that causes a genetic disease.

Explain your choice.

---**[2]**

iii. Suggest how the interdisciplinary field of *bioinformatics* may be useful in determining whether a newly-sequenced allele causes a genetic disease.

---**[2]**

13. Lungs are the specialised gas exchange surfaces in mammals. Dogs are mammals.

A disease called canine pulmonary fibrosis (CPF) can affect lung function in dogs. CPF can reduce the tidal volume of a dog's lungs.

i. The West Highland Terrier develops CPF more often than other breeds of dog.

The lung function of a West Highland Terrier was tested. At rest, its ventilation rate was 1.44 dm^3 min^{-1} and its breathing rate was 24 breaths min^{-1}.

Calculate the tidal volume of the West Highland Terrier in cm^3.

Tidal volume = ... cm^3 **[1]**

ii. Explain how the high occurrence of CPF in West Highland Terriers could have been a result of artificial selection.

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[1]

iii. Explain how DNA sequencing could help scientists understand how the West Highland Terrier's genes affect its probability of developing CPF.

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[2]

iv. Another disease that affects dogs is caused by parvovirus. Dogs can be vaccinated against parvovirus at six weeks of age.

Suggest what the parvovirus vaccine is likely to contain.

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[1]

v. Dogs need a booster vaccination against parvovirus when they are one year old.

Explain why a booster vaccination is needed.

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[1]

14. DNA profiling uses techniques to separate lengths of DNA to produce a profile that is unique to each individual.

Explain why only selected sections of non-coding DNA are used when profiling a human.

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[3]

15. Agammaglobulinemia and Vici syndrome are both genetic diseases.

DNA profiling can be used to analyse the risk of inheriting conditions such as agammaglobulinemia and Vici syndrome.

i. To produce a DNA profile, DNA first needs to be purified.

Explain why a protease enzyme is added to the mixture during the DNA purification process.

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[1]

ii. DNA samples can be amplified using the polymerase chain reaction (PCR).

In theory, how many fragments of DNA might be present after 12 cycles of PCR?

Assume one DNA fragment was present at the beginning of the PCR process. Represent your answer as a $\log_{10}$ value.

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fragments **[2]**

iii. Suggest why the figure you calculated in (ii) may not be achieved in practice.

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[1]

iv. State the name of the enzyme used in PCR to synthesise new DNA strands.

---

[1]

v. DNA fragments are separated to produce a DNA profile using electrophoresis.

A student wrote the following description of the electrophoresis procedure:

> **We will set up an agarose gel plate and place the DNA samples in the wells at the cathode. Voltage will be passed through the gel for one minute. The gel will then be placed in purified water and we will be able to see the banding pattern of each DNA sample.**

Describe **two** changes you would make to the student's procedure and explain how these changes would improve electrophoresis.

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_____

_____

_____

_____

_____ **[2]**

16. Tissue traces from a crime scene often need to be identified. DNA from the tissue is 'amplified' by the polymerase chain reaction (PCR) to get samples large enough for further analysis.

Modern PCR technique uses DNA polymerase from the bacterium *Thermus aquaticus*. Why is this enzyme chosen?

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_____

_____ **[2]**

17. Fig. 22 shows four nucleotides.

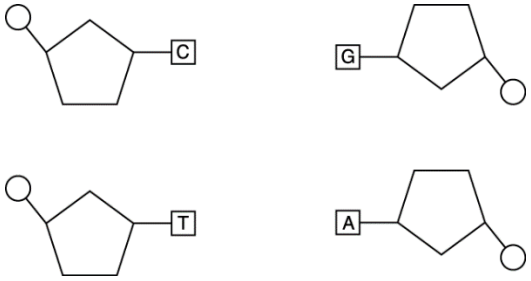

Fig. 22

i. **On Fig. 22**, use the letter **R** to label a bond that would be made by the action of a ligase enzyme.

[1]

ii. **On Fig. 22**, use the letter **P** to label a bond that would be broken during the hottest step of the PCR reaction.

[1]

18(a). DNA fragments can be separated using electrophoresis.

Fig. 3.1 shows the result of electrophoresis of several DNA samples.

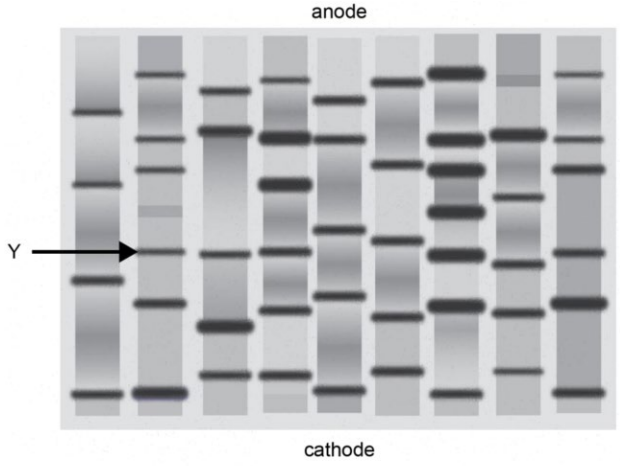

Fig. 3.1

i. Describe how DNA can be visualised after electrophoresis has been completed.

---**[2]**

ii. Place a cross (X) **on Fig. 3.1** to indicate the position of a fragment of DNA with a mass greater than the DNA band labelled Y.

[1]

(b).

i. Mixtures of proteins can also be separated by electrophoresis.

- Proteins are heated before being placed in the electrophoresis gel.
- The gel contains a substance called SDS, which has a negative charge.
- SDS binds to proteins.

Suggest why proteins are heated before being placed in the electrophoresis gel.

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_____

_____ **[1]**

ii. Suggest why the binding of SDS to proteins is necessary for protein electrophoresis.

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_____ **[2]**

19. An Iron Age farm was excavated by archaeologists. Some DNA was recovered from the tooth of an animal thought to be a type of domesticated milk cow.

A farmer keeps rare breed cows similar to those farmed on the Iron Age farm. DNA from the cows was obtained.

What technique would you plan to use, to compare digested and amplified fragments from the two DNA samples?

_____ **[1]**

20. The European corn borer moth, *Ostrinia nubilalis,* is a pest of agriculture. Its larvae develop inside maize stems and eat the contents, weakening the stems so that the plants collapse.

The bacterium *Bacillus thuringiensis* ('Bt') produces a protein that poisons the larvae of moths and butterflies. This protein can be isolated from cultures of Bt and packaged in fluids to be sprayed on the surface of plants.

The gene coding for the toxic protein has also been isolated. It has been incorporated into a genetically modified strain of maize called Bt corn. This makes the plant tissues poisonous to the corn borer moth.

Consider the statement:

'Genetically modified plants and animals should be classed as new species'.

Outline **one** experiment or investigation that would provide evidence to support or contradict the statement.

_____

_____

_____

_____

_____

_____ **[3]**

21. When sequencing DNA, fragments of DNA are separated by electrophoresis.

Describe **three** differences between the process of thin layer chromatography and the form of electrophoresis used to sequence DNA.

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_____

_____

_____ **[3]**

22(a). Potatoes often suffer bruising, which reduces their value as a food crop.

A variety of crop potato that does not bruise has been developed using a technique called gene silencing.

Scientists carry out gene silencing by inserting small sequences of RNA into potato cells. These RNA sequences are complementary to mRNA from genes responsible for bruising.

Use this information to suggest why the technique is called 'gene silencing'.

_____

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_____

_____

_____

_____ **[2]**

(b). *Scientists have genetically engineered many different species of plant.

Aubergine plants, *Solanum melongena*, can suffer damage from moth larvae.

Scientists have produced a variety of aubergine that is resistant to moth larvae. To create the resistance, scientists transferred a gene from the *Bacillus thuringiensis* bacterium.

Describe the process the scientists could have used to produce the pest-resistant aubergines.

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_____ **[6]**

23(a). Fig. 21 shows some of the steps involved in producing a genetically modified bacterium.

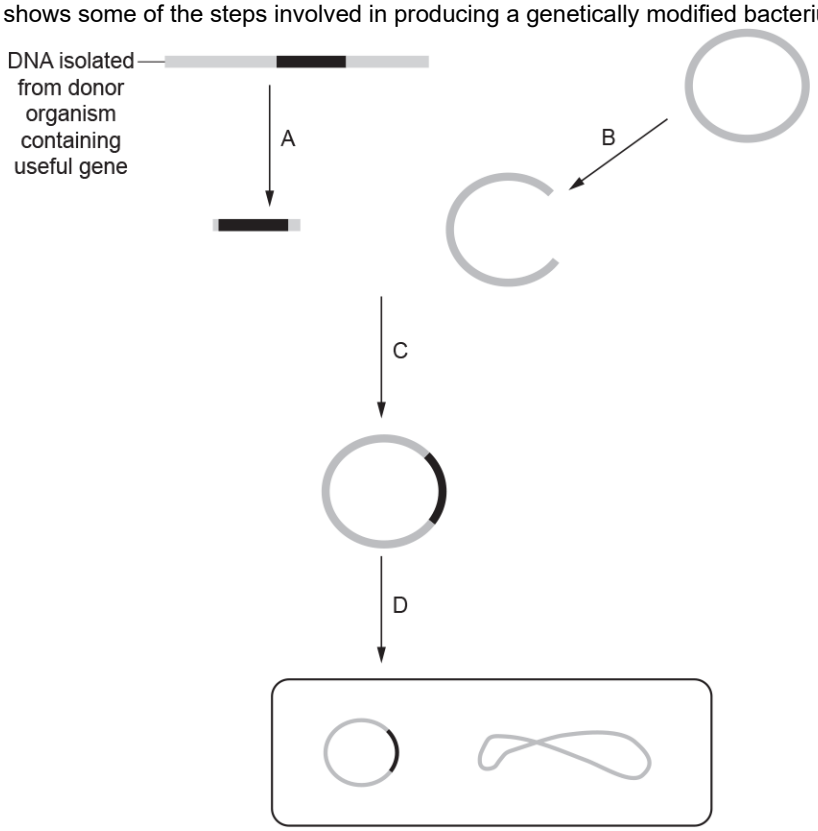

Fig. 21

The following passage describes steps A and B. Complete the passage using the most appropriate terms.

A gene is cut from the DNA of the donor organism using a …… …… .

The …… enzyme is used to cut open a small piece of bacterial DNA so that the base sequences at the end of each piece of DNA are …… .

[3]

(b). Describe the **events** that are taking place at the step labelled C.

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_____

_____ **[3]**

(c). Step D results in a transformed bacterium.

Many individual bacteria are not transformed successfully during this procedure. Explain how scientists can determine the success of step D in this procedure.

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_____

_____ **[3]**

(d). Bacteria can be genetically modified to produce human insulin.
The process is similar to that shown in **Fig. 21** with some differences.
First, instead of isolating DNA that contains the insulin gene, mRNA that codes for insulin is extracted from human pancreas cells.

What needs to be done with the mRNA in order for the rest of the genetic modification to be completed?

_____

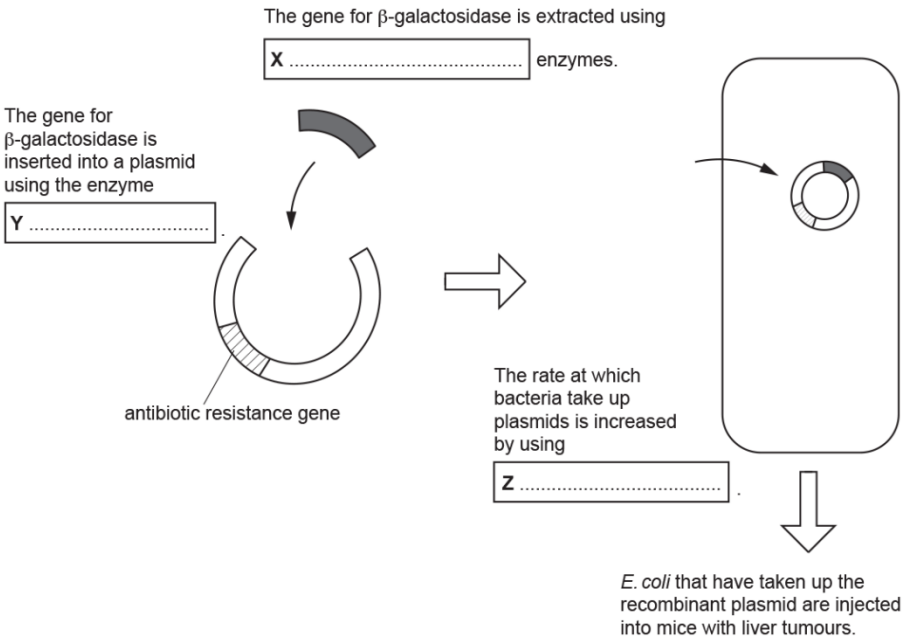
_____

_____

_____ **[2]**

24. Bacteria such as *E. coli* can be genetically engineered for use in medical science.

An example of the genetic engineering of *E. coli* is shown in the diagram below.



i. Complete the diagram above by writing the missing words or phrases in the boxes labelled **X**, **Y** and **Z**.

Answer **on** the diagram **[3]**

ii. Suggest why the scientists used a plasmid that contained an antibiotic resistance gene.

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[1]

iii. The scientists observed the following:

- 1 in 400 bacteria took up the plasmid
- 1 in 1000 of the plasmids taken up by bacteria contained the β-galactosidase gene.

Calculate the percentage of bacteria that contained the β-galactosidase gene.

percentage of bacteria = ... % **[2]**

iv. A technique called quantitative PCR is used to check that the *E. coli* population is growing on the mice liver tumours rather than on healthy tissue.

Suggest how the scientists could use PCR to **compare** *E. coli* growth rates on cancerous liver tissue and healthy tissue.

[2]

v. Some people think that the genetic engineering of certain organisms is unethical.

However, there are very few ethical concerns about the genetic engineering of bacteria such as *E. coli*.

Suggest why there are very few ethical concerns about the genetic engineering of *E. coli*.

[1]

25. Some people are concerned about genetic modification.

State one valid concern that people have about the genetic modification of **bacteria**.

[1]

26(a). * Some students investigated the different ways of protecting maize plants against the corn borer moth. In each of **three** separate but close-together square plots, in the same field, they planted several hundred maize seedlings.

Plot A: untreated (control).

Plot B: sprayed daily with Bt toxin.

Plot C: the seedlings planted were genetically modified Bt corn.

On the first day of each week, one student would walk around the edge of a plot and count the number of maize plants that had collapsed in that plot. Each plot had a student responsible for counting. The results are shown in **Table 20.1.**

	Number of maize plants collapsed since the last weekly count		
Week number	**Plot A**	**Plot B**	**Plot C**
1	0	0	1
2	0	0	0
3	18	22	21
4	0	0	0
5	5	1	0
6	14	11	12
7	5	2	1
8	12	0	1
9	17	1	0
10	30	6	0
11	32	13	1
12	41	17	0
13	38	26	1
14	47	31	1
15	50	44	2
16	49	47	0

Table 20.1

The students' tutor raised a number of concerns about the investigation. In summary:

- **The methods were not a valid test of what was being investigated.**
- **The results may not be accurate.**
- **Some variables were not controlled.**

Explain why these concerns are justified and suggest improvements to the investigation.

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_____ **[6]**

(b). A farmer wants to increase the yield of maize.

A friend recommends planting genetically-modified Bt corn as it would be more effective against European corn borer larvae rather than spraying unmodified corn with Bt toxin.

Which method would you recommend to the farmer? Justify your answer.

_____

_____ **[1]**

27. Genes isolated from DNA can be used in gene therapy.

Cystic fibrosis (CF) is a disease that could be treated using gene therapy.

Healthy individuals have a gene that codes for a channel protein, called CFTR, found in the plasma membrane of a variety of cells, including those lining the airways of the lungs.

People suffering from CF have two copies of a recessive allele and so their cells do not synthesise the correct channel protein.

The allele that codes for the functioning CFTR protein can be inserted into the DNA of CF sufferers. The cells can then synthesise the correct CFTR protein and function as normal.

i. The treatment of cystic fibrosis is described as **somatic** gene therapy. Another type of gene therapy is known as **germ-line** gene therapy.

Complete the table below to show **three** differences between somatic gene therapy and germ-line gene therapy.

Somatic	Germ-line

[3]

ii. Some attempts at gene therapy have resulted in changes to the functioning of other genes.

Explain how inserting a new gene into a chromosome could affect the functioning of other genes in that chromosome.

---**[2]**

iii. CF occurs when individuals have two copies of a recessive allele.

Huntington's disease is a lethal disease caused by a dominant allele that codes for the protein huntingtin.

Suggest why gene therapy is unlikely to work as a treatment for Huntington's disease.

---**[1]**