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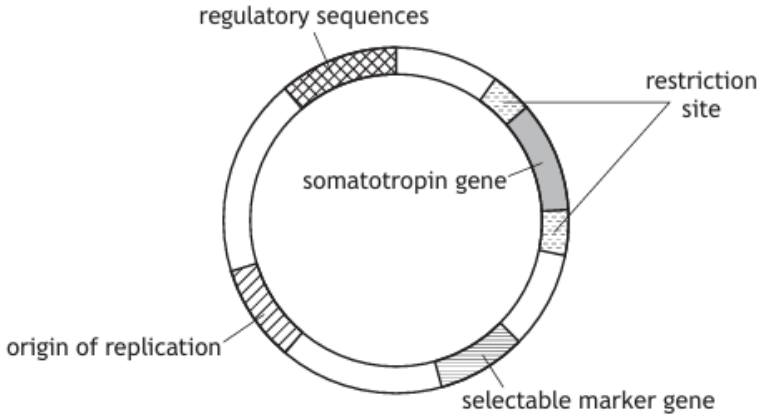
1. A pharmaceutical company used recombinant DNA technology to produce genetically modified bacteria that synthesised the human growth hormone somatotropin.

The diagram shows the modified plasmid that was used to transform the bacteria.



The transformed bacteria were resistant to the antibiotic ampicillin.

Which feature of the modified plasmid is responsible for this resistance?

A Regulatory sequences
B Somatotropin gene
C Origin of replication
D Selectable marker gene

2. Which statement about recombinant DNA technology is **not** correct?

A Plasmids are examples of vectors.
B Ligase cuts open plasmids and cuts specific genes out of chromosomes.
C Recombinant bacteria may result in proteins that are folded incorrectly.
D Artificial chromosomes are used when larger fragments of DNA are inserted.

3. An experiment was carried out to investigate the effectiveness of a sunscreen on the survival of yeast cells.

Yeast was added to a Petri dish containing agar. Sunscreen was spread across the lid before the dish was exposed to UV light.

A valid conclusion, relating to the aim, could be drawn by setting up a control experiment without

A yeast
B sunscreen
C yeast and no exposure to UV light
D sunscreen and no exposure to UV light.

4. Using recombinant DNA technology, the bacterium *E. coli* can be modified so that it can produce human insulin. The following steps are involved.

1 Culture large quantities of *E. coli* in nutrient medium.
2 Insert human insulin gene into *E. coli* plasmid DNA.
3 Cut insulin gene from human chromosome using enzymes.
4 Extract insulin from culture medium.

The correct order of these steps is

A 3, 2, 1, 4
B 3, 1, 2, 4
C 1, 4, 3, 2
D 1, 2, 3, 4.

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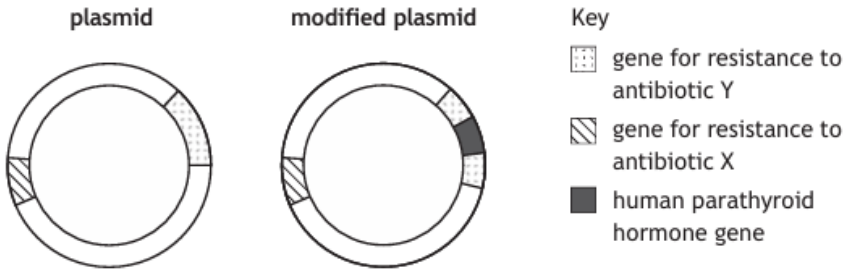
5. Human parathyroid hormone can be produced in bacterial cells by recombinant DNA technology. The diagram shows a plasmid before and after being modified by inserting the human parathyroid hormone gene. This disrupted the gene for resistance to antibiotic Y.



Bacterial cells were incubated with the modified plasmids and grown on nutrient media in three different Petri dishes.

The table shows the nutrient media used in each Petri dish.

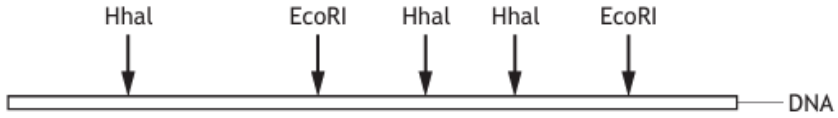
Petri dish	Nutrient medium
1	with antibiotic X only
2	with antibiotic Y only
3	with antibiotics X and Y

In which Petri dish(es) would the human parathyroid hormone be produced?

A 1 only
B 2 only
C 1 and 3 only
D 2 and 3 only

6. During recombinant DNA technology, restriction endonucleases are used to cut DNA into fragments. Each endonuclease recognises a specific DNA sequence called a restriction site.

The diagram shows a section of DNA and the restriction sites for two different endonucleases, EcoRI and HhaI.



Identify the number of DNA fragments that would be produced after treating this section of DNA with the restriction endonucleases shown in the table.

	Restriction endonucleases	Number of DNA fragments
A	EcoRI	2
B	HhaI	3
C	EcoRI + HhaI	5
D	EcoRI + HhaI	6

7. Using recombinant DNA technology, a culture of the bacteria species *Micrococcus luteus* was genetically modified with a plasmid containing a gene for a human protein. The protein was synthesised by the genetically modified bacteria, but it failed to fold correctly.

Which of the following changes to this procedure may lead to a correctly folded protein being produced?

A Use a different species of bacteria
B Use yeast cells rather than bacteria
C Insert an artificial chromosome instead of a plasmid
D Insert a regulatory sequence into the plasmid

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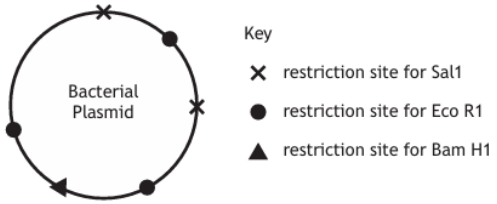
8. The diagram shows a bacterial plasmid with restriction sites for three different restriction endonucleases, Sal1, Eco R1 and Bam H1.



Which row in the table identifies the number of fragments produced if the plasmid was cut with the combinations of restriction endonucleases shown?

	Combination	
	Sal1 and Bam H1	*Sal1 and Eco R1*
A	3	4
B	3	5
C	4	4
D	4	5

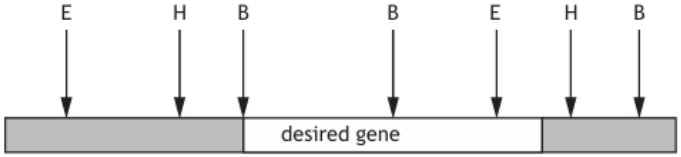
9. The diagram below shows part of a DNA strand and the sites at which three different endonucleases can cut the strand.

- EcoRI endonuclease
- HindIII endonuclease
- SmaI endonuclease

Which line in the table below identifies correctly the number of DNA fragments which would be obtained if this DNA strand was cut with the endonuclease(s) shown in the table?

	Endonucleases			
	EcoRI	*SmaI*	*HindIII*	*EcoRI and HindIII*
A	2	2	1	3
B	2	3	2	3
C	4	2	1	5
D	4	3	2	5

10. DNA recombinant technology can involve the insertion of a desired gene into a plasmid.
The diagram shows restriction sites on a chromosome containing the desired gene.
The restriction sites are H (HindIII), E (EcoR1) and B (BamH1).



The desired gene should be removed with

A HindIII
B EcoR1
C BamH1
D BamH1 and HindIII.

11. Plasmids containing the gene for frost resistance (FRO) and a gene for resistance to the antibiotic ampicillin were incubated with a suitably prepared culture of *E. coli*. The bacteria were then plated out on nutrient agar that either contained ampicillin (+ amp) or did not (– amp).

Which line in the table below shows the status of the bacteria and the agar that would **not** result in colonies?

	Plasmid taken up	*Agar type*
A	Yes	– amp
B	No	+ amp
C	Yes	+ amp
D	No	– amp

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12

In the laboratory, bacteria can be transformed by introducing plasmids that carry genes for new functions. The *transformation efficiency* of the procedure measures how many bacteria take up the plasmid in relation to the mass of DNA used.

$$\text{Transformation efficiency} = \frac{\text{Number of colonies formed}}{\text{Mass of plasmid DNA (µg)}}$$

10 µl of a solution of plasmid DNA at a concentration of 0·08 µg/µl resulted in 480 transformed bacteria. The transformation efficiency is

A 48

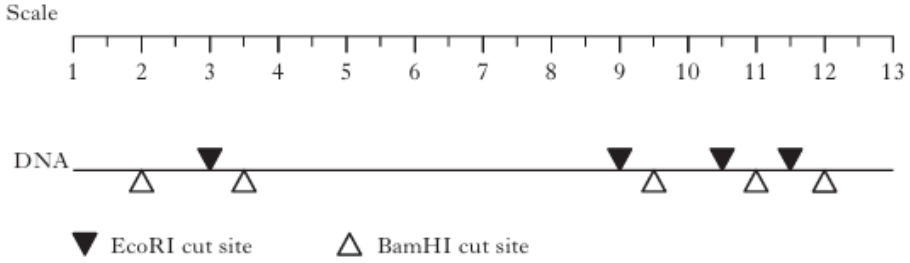
B 384

C 600

D 6000.

13

A piece of DNA is cut into fragments using the restriction endonucleases BamHI and EcoRI at the sites indicated in the diagram below.



How many bands would be observed following separation of the fragments by electrophoresis?

A 3

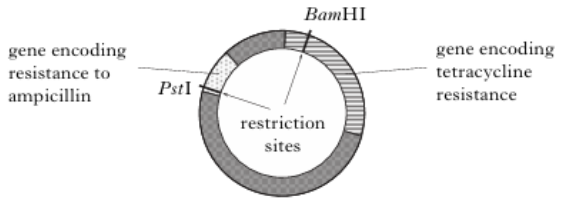
B 5

C 6

D 10

14.

The diagram below shows the restriction enzyme sites in a plasmid that carries the genes for resistance to the antibiotics ampicillin and tetracycline.

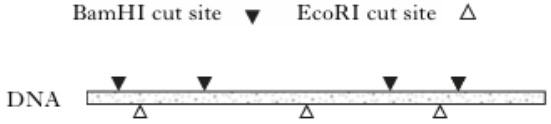


Which line in the table below identifies correctly the antibiotic resistance that would remain when a gene is inserted at these restriction enzyme sites?

	Gene inserted into restriction enzyme site	*Antibiotic resistance remaining*
A	*Bam*HI	tetracycline and ampicillin
B	*Pst*I	ampicillin
C	*Pst*I	tetracycline and ampicillin
D	*Bam*HI	ampicillin

15.

A length of DNA is cut into fragments by the restriction enzymes BamHI and EcoRI.



Which of the following gives the correct number of DNA fragments obtained?

	DNA cut by BamHI only	*DNA cut by EcoRI only*	*DNA cut by both BamHI and EcoRI*
A	5	4	8
B	4	5	8
C	5	4	9
D	4	5	9

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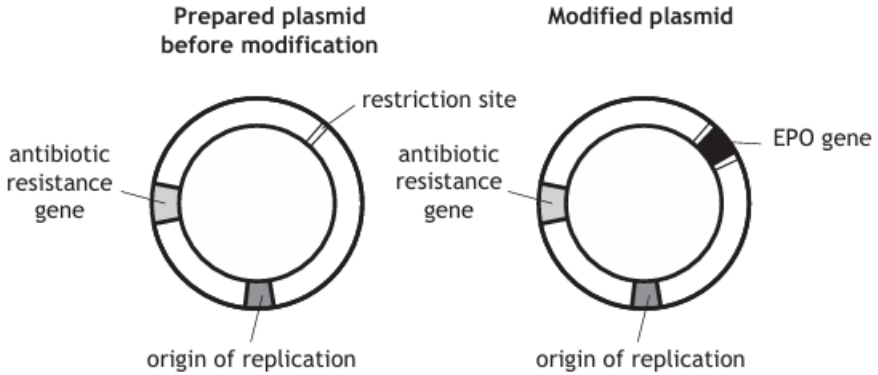
MARKS

1. Erythropoietin (EPO) is a protein synthesised in the kidneys which is involved in red blood cell production. Some individuals with kidney disease have low red blood cell counts and can be treated with EPO.

EPO can be produced by recombinant DNA technology in which the human EPO gene was inserted into a specially prepared bacterial plasmid.

The diagram below shows the prepared bacterial plasmid before and after it was modified by the insertion of a human EPO gene.



(a) Explain the importance of removing the EPO gene from a human chromosome with the **same** restriction endonuclease that was used to open the bacterial plasmid. **1**

(b) Name the enzyme used to seal the EPO gene into the bacterial plasmid. **1**

(c) Modified plasmids were mixed with bacteria. Some bacterial cells were transformed by taking up the modified plasmids but others were not.

Use information from the diagram to suggest how a culture containing only the transformed bacteria was obtained. **1**

(d) Identify the section of the modified plasmid shown in the diagram which ensured that it could be copied and passed to daughter cells when transformed bacteria divided. **1**

(e) The EPO protein produced by the transformed bacteria is inactive.

(i) Suggest a reason why bacteria produce EPO protein which is inactive. **1**

(ii) Suggest how recombinant DNA technology could be used to produce an active form of the EPO protein. **1**

2. Name a process by which a wild strain of a micro-organism can be improved to increase the yield of a desired product. **1**

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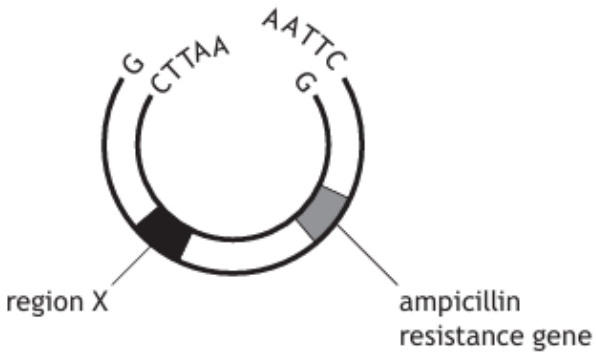
3. The diagram shows some features of a plasmid which has been cut open by restriction endonuclease to allow a gene from a donor chromosome to b inserted.



The DNA recognition sites for three restriction endonucleases, *E coR1*, *BamH* and *HindIII*, are shown in the table. The arrows indicate where each restricti endonuclease cuts the DNA sequence.

Restriction endonuclease	*DNA sequence recognised*
E coR1	G↓AATTC CTTAA↑G
BamH1	G↓GATCC CCTAG↑G
HindIII	A↓AGCTT TTCGA↑A

(a) (i) A restriction endonuclease was used to remove a gene from a donor chromosome.

Use information from the diagram and the table to identify the restriction endonuclease which would be used to allow the gene to be inserted into the plasmid.

Give a reason for your answer. **2**

Restriction endonuclease _____

Reason _____

_____

(ii) Name the enzyme which would be used to seal the gene into the plasmid. **1**

_____

(iii) A culture of bacterial cells, 20% of which had taken up this modified plasmid, were grown on a nutrient agar plate. The plate was incubated and 250 colonies of this bacteria grew.

Predict the number of colonies which would have been expected to grow if the nutrient agar plate had contained the antibiotic ampicillin. **1**

Space for calculation

_____

(b) Name region X, shown in the diagram, which ensured that the modified plasmid would be passed on to daughter cells. **1**

_____

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4. An investigation was carried out into the effect of increasing time of exposure to UV light on the survival of wild type (WT) and mutant (M) yeast cells.

Each type of yeast cell was grown in separate liquid media at 30 °C for 24 hours, diluted and plated onto separate agar plates. They were then exposed to a UV light source for between 0 and 30 seconds in a darkened room.

The plates were incubated for four days at 20 °C and the number of yeast colonies that had grown was counted. Each colony grew from a single cell.

The results are shown in the table.

Time of exposure to UV light (seconds)	Number of yeast colonies	
	WT	M
0	360	400
5	210	120
10	90	25
15	45	10
20	20	0
30	10	0

(a) (i) State an independent variable in this experiment. **1**

_____

(ii) Suggest why exposure to UV light was carried out in a darkened room. **1**

_____

_____

b) Draw **one** conclusion from the results of this investigation. **1**

_____

_____

c) Sunscreen lotions can protect cells from UV damage.

Suggest how the investigation could be modified to test the effectiveness of a sunscreen lotion using M yeast as model cells. **2**

_____

_____

_____

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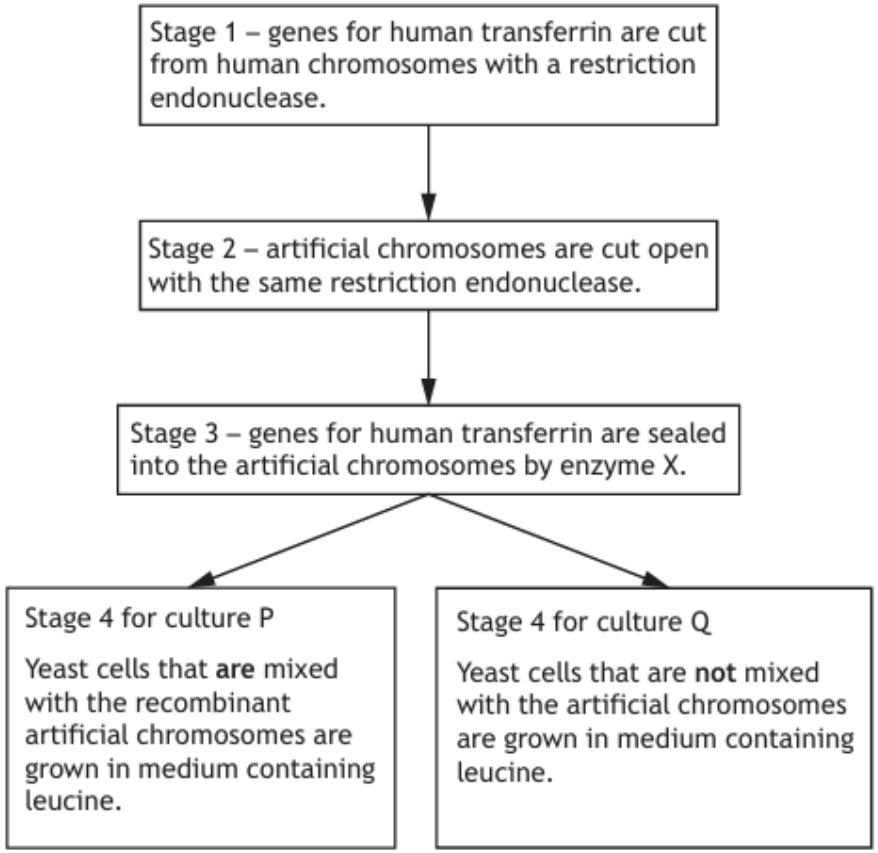
5. Transferrin is a protein used to treat the blood disorder anaemia in humans.

Artificial chromosomes are used in recombinant DNA technology to genetically modify yeast cells to produce transferrin.

The strain of yeast cells used cannot synthesise the amino acid leucine, which is necessary for protein synthesis and growth of the yeast.

Some stages of this recombinant DNA technology process are shown in the diagra



(a) (i) State the term used to describe the artificial chromosome that carries the human gene into yeast cells. **1**

(ii) State why an artificial chromosome rather than a plasmid may be used in recombinant DNA technology. **1**

(b) Explain why the same restriction endonuclease is used in stages 1 and 2. **1**

(c) Name enzyme X used in stage 3. **1**

(d) The artificial chromosome used contains a selectable marker gene that only allows transformed yeast cells to synthesise leucine.

After stage 4, cultures P and Q were transferred to separate plates containing solid medium without leucine.

After incubation, yeast cells only grew on the plate containing culture P.

Explain this result. **2**

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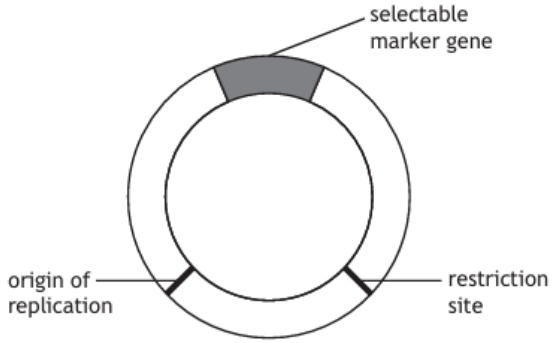
6. Recombinant DNA technology involves the use of plasmids to transfer desired genes into microbial cells.

The diagram shows some key features of a plasmid used in recombinant DNA technology.



(a) (i) State the term used to describe the role of the plasmid in recombinant DNA technology. 1

_____

(ii) Describe the function of the origin of replication. 1

_____

_____

(b) Name the type of enzyme used to remove a desired gene from a chromosome and cut open the plasmid. 1

_____

(c) When bacteria take up recombinant plasmids they are said to be transformed.

The selectable marker gene on the plasmid allows transformed bacteria to be identified.

Give an example of a selectable marker gene and explain how it allows transformed bacteria to be identified. 2

Selectable marker gene _____

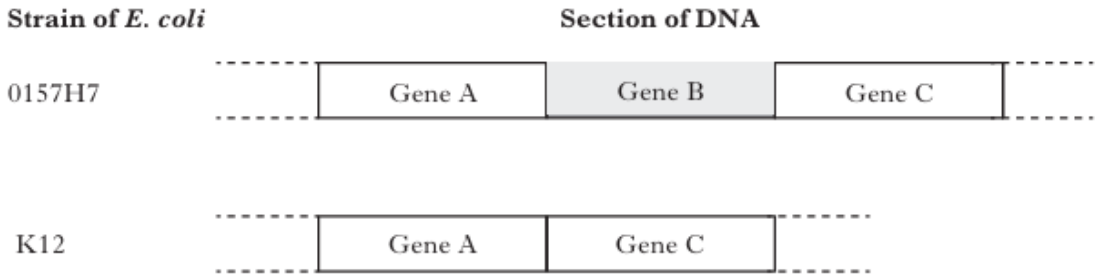
Explanation _____

_____

7. *Escherichia coli* is a species of bacteria found in the lower intestines of humans.

Sections of the DNA of two strains of *E. coli* are shown below.

Gene B codes for a protein known as Shiga toxin which can cause serious food poisoning in humans.



(*a*) The gene for Shiga toxin has been acquired by *E. coli* 0157H7.

Give a method by which the bacterial strain could have acquired this gene.

_____

_____ 1

(*b*) (i) *E. coli* K12 is routinely used in recombinant DNA technology. Explain why *E. coli* K12 is used whereas *E. coli* 0157H7 is not.

_____

_____ 1

(ii) Bacteria are used in recombinant DNA technology.

Explain why animal DNA which has been transferred to bacteria may produce proteins which are not functional.

_____

_____ 1

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Mark:

8. **Diagram 1** below shows a plasmid that contains two genes for antibiotic resistance. **Diagram 2** shows the same plasmid after modification by the insertion of a gene from another organism.

Diagram 1
Plasmid before modification

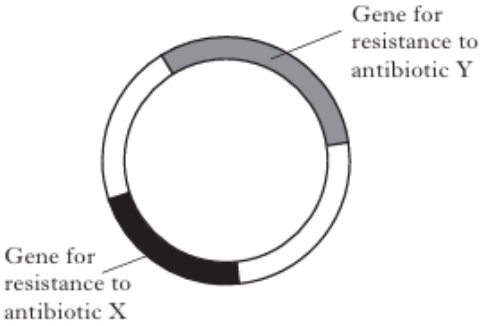
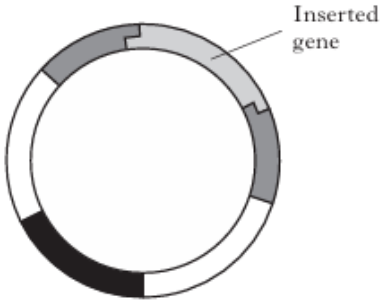


Diagram 2
Plasmid after modification



(*a*) Two different enzymes are required to produce the modified plasmid.

Complete the table below by:

(i) naming the enzyme which cuts plasmids at specific restriction sites; **1**

(ii) naming the second enzyme required to modify plasmids; **1**

(iii) describing the function of the second enzyme. **1**

Enzyme required to produce modified plasmids	*Function of enzyme in the modification process*
	Cuts plasmids at specific restriction sites

(*b*) Bacteria took up the modified plasmids.

Explain why these bacteria will not have resistance to antibiotic Y.

_____

_____

_____ **1**

9. Write notes on plasmids as vectors in recombinant DNA technology. **4**

1

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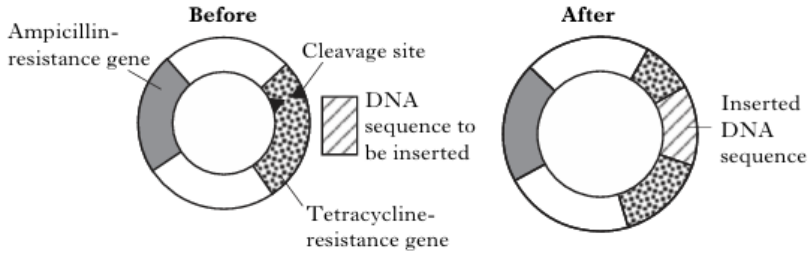
10 Genetic manipulation of bacteria can be achieved by the introduction of plasmids containing DNA sequences from other organisms. The diagram shows a plasmid containing resistance genes for the antibiotics ampicillin and tetracycline, before and after the insertion of a DNA sequence.

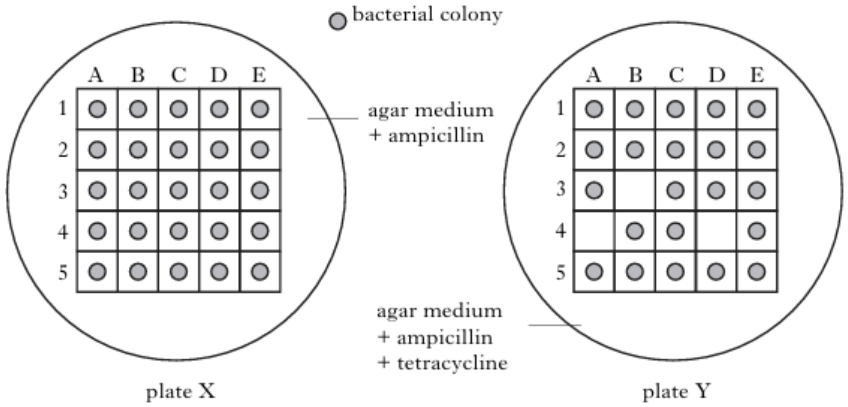


There are two possible problems with this approach:

(i) only some of the plasmids take up the DNA sequence;

(ii) only a small percentage of bacterial cells take up a plasmid of any kind.

Bacteria containing plasmids with the desirable DNA sequence can be selected by culturing them on media containing antibiotics. The same colonies are plated in the same grid positions on the two plates shown below.



(*a*) What is the effect of inserting the DNA sequence into the tetracycline-resistance gene? **1**

(*b*) Explain why the colonies A4, B3 and D4 from plate Y would be selected for further analysis. **2**

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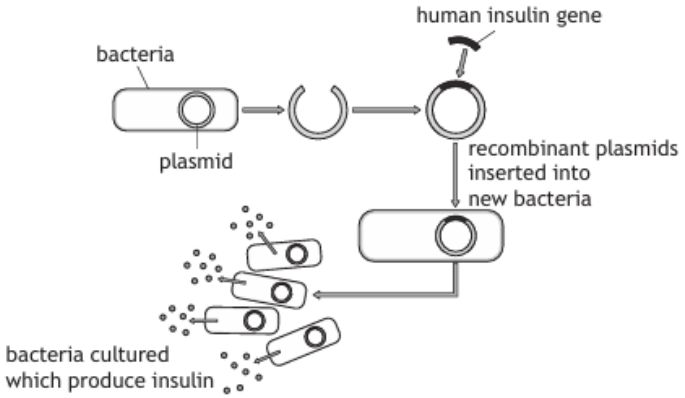
MARKS

11. The diagram below shows how a human gene can be inserted into bacteria to produce human insulin using recombinant DNA technology.



(a) Name **one** enzyme used in this process and state its function. **2**

Name _____

Function _____

(b) (i) The recombinant plasmid also contains a gene for resistance to the antibiotic, ampicillin.

Describe a procedure which would allow only cells containing the recombinant plasmid to be selected. **2**

_____

_____

_____

(ii) Plasmids with these antibiotic resistance genes have been passed to other bacterial species by horizontal transfer.

Describe the process of horizontal transfer. **1**

2. _____

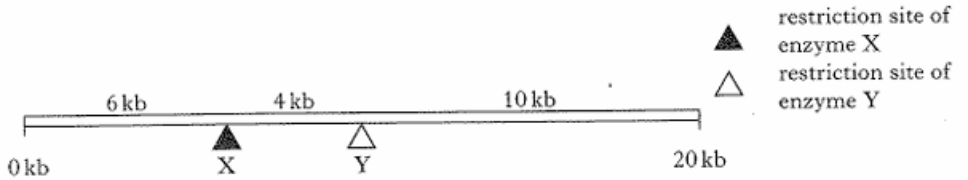
_____

(c) When culturing the bacteria which produce insulin, sterile conditions are maintained.

Explain why this is important. **1**

Marks

12 Restriction enzymes cut strands of DNA double helix at specific sites called *restriction sites*. The effect of using the enzymes is to cut the DNA into fragments, which can be separated by electrophoresis. *Restriction maps* can be created from the fragment information to show the relative positions of the restriction sites in kilobase (kb) lengths, as shown in the following example.



(*a*) What sizes of fragment would be produced if the 20 kb piece of DNA shown in the map above was cut with enzyme X only? **1**

_____

(*b*) The table shows the number and sizes of fragments from a digest of a 10 kb DNA sample using two restriction enzymes, A and B.

Enzymes added	*Fragment size* (kb)		
A only	8	2	–
B only	7	3	–
A and B together	5	3	2

Draw a restriction map for the digest, labelling where the restriction sites are in relation to each other. **1**

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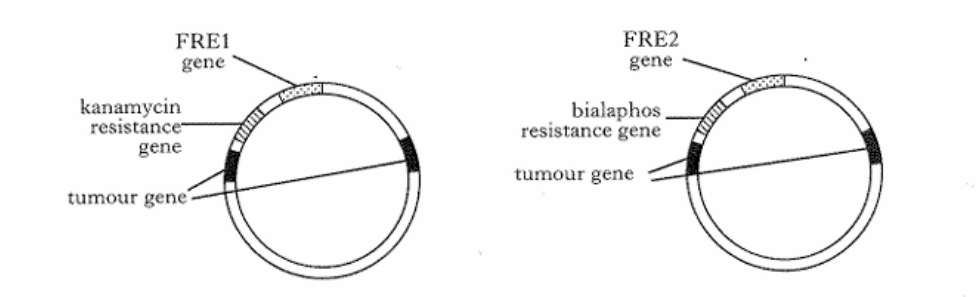
13 Agrobacterium Tumefaciens is a bacteria which can infect plants via its tumour inducing (Ti) plasmids causing tumour like growths at the base of the plant stem.

The aim of the investigation was to determine the effect of iron uptake genes on genetically engineered tobacco plants' growth.

Ti plasmids can be modified to inhibit tumour formation and FRE 1 or FRE 2 genes added which facilitate plant iron uptake from the soil via the plant roots. Iron is an important nutrient for plant growth as it is needed for chlorophyll formation.

Modified Ti plasmids with FRE 1 or 2 genes were incubated with tobacco plant cells in media to produce transformed tobacco plants. To check the success of the transformation process, Ti plasmids had kanamycin or bialaphos resistance genes added and grown in appropriate media.



Two groups of plants were produced to begin with, one with the FRE 1 gene and the other with the FRE 2 gene. These plants were then bred together to produced a third group with both FRE 1 and FRE 2 genes. A control group was also tested at the same test.

The results of the iron concentration of tobacco plant leaf tissues grown in high and low iron soil are shown below.

	Iron concentration (µg/g dry mass)	
Plant Group	*"High" Iron*	*"Low" Iron*
Controls	129	26
KA (FRE1)	154	31
BI (FRE2)	199	43
KA+BI (FRE1+2)	223	41

a) Describe how the control group was set up.

_____

_____

b) When tobacco cells take up the modified Ti plasmid they are said to be transformed.
For the FRE 2 Ti plasmid state the selectable marker and explain how it allows detection of only transformed tobacco plants.

Selectable marker _____

Explanation _____

_____

C) Draw one conclusion from the results of the investigation

_____

_____

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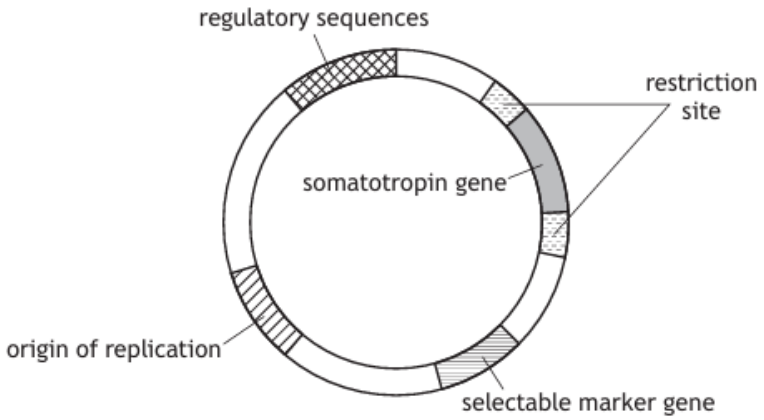
1. A pharmaceutical company used recombinant DNA technology to produce genetically modified bacteria that synthesised the human growth hormone somatotropin.

The diagram shows the modified plasmid that was used to transform the bacteria.



The transformed bacteria were resistant to the antibiotic ampicillin.

Which feature of the modified plasmid is responsible for this resistance?

A Regulatory sequences
B Somatotropin gene
C Origin of replication
D Selectable marker gene

2. Which statement about recombinant DNA technology is **not** correct?

A Plasmids are examples of vectors.
B Ligase cuts open plasmids and cuts specific genes out of chromosomes.
C Recombinant bacteria may result in proteins that are folded incorrectly.
D Artificial chromosomes are used when larger fragments of DNA are inserted.

3. An experiment was carried out to investigate the effectiveness of a sunscreen on the survival of yeast cells.

Yeast was added to a Petri dish containing agar. Sunscreen was spread across the lid before the dish was exposed to UV light.

A valid conclusion, relating to the aim, could be drawn by setting up a control experiment without

A yeast
B sunscreen
C yeast and no exposure to UV light
D sunscreen and no exposure to UV light.

4. Using recombinant DNA technology, the bacterium *E. coli* can be modified so that it can produce human insulin. The following steps are involved.

1 Culture large quantities of *E. coli* in nutrient medium.
2 Insert human insulin gene into *E. coli* plasmid DNA.
3 Cut insulin gene from human chromosome using enzymes.
4 Extract insulin from culture medium.

The correct order of these steps is

A 3, 2, 1, 4
B 3, 1, 2, 4
C 1, 4, 3, 2
D 1, 2, 3, 4.

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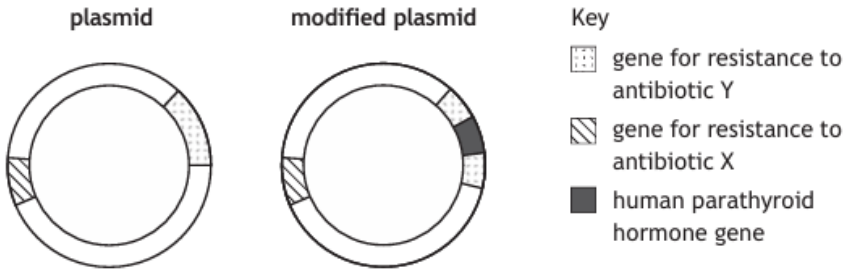
5. Human parathyroid hormone can be produced in bacterial cells by recombinant DNA technology. The diagram shows a plasmid before and after being modified by inserting the human parathyroid hormone gene. This disrupted the gene for resistance to antibiotic Y.



Bacterial cells were incubated with the modified plasmids and grown on nutrient media in three different Petri dishes.

The table shows the nutrient media used in each Petri dish.

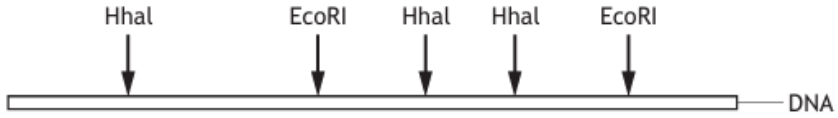
Petri dish	Nutrient medium
1	with antibiotic X only
2	with antibiotic Y only
3	with antibiotics X and Y

In which Petri dish(es) would the human parathyroid hormone be produced?

A 1 only
B 2 only
C 1 and 3 only
D 2 and 3 only

6. During recombinant DNA technology, restriction endonucleases are used to cut DNA into fragments. Each endonuclease recognises a specific DNA sequence called a restriction site.

The diagram shows a section of DNA and the restriction sites for two different endonucleases, EcoRI and HhaI.



Identify the number of DNA fragments that would be produced after treating this section of DNA with the restriction endonucleases shown in the table.

	Restriction endonucleases	Number of DNA fragments
A	EcoRI	2
B	HhaI	3
C	EcoRI + HhaI	5
D	EcoRI + HhaI	6

7. Using recombinant DNA technology, a culture of the bacteria species *Micrococcus luteus* was genetically modified with a plasmid containing a gene for a human protein. The protein was synthesised by the genetically modified bacteria, but it failed to fold correctly.

Which of the following changes to this procedure may lead to a correctly folded protein being produced?

A Use a different species of bacteria
B Use yeast cells rather than bacteria
C Insert an artificial chromosome instead of a plasmid
D Insert a regulatory sequence into the plasmid

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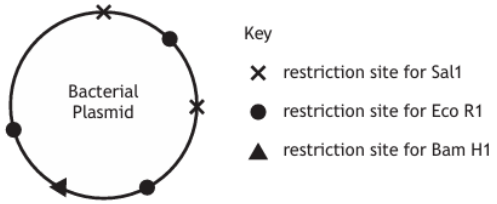
8. The diagram shows a bacterial plasmid with restriction sites for three different restriction endonucleases, Sal1, Eco R1 and Bam H1.



Which row in the table identifies the number of fragments produced if the plasmid was cut with the combinations of restriction endonucleases shown?

	Combination	
	Sal1 and Bam H1	*Sal1 and Eco R1*
A	3	4
B	3	5
C	4	4
D	4	5

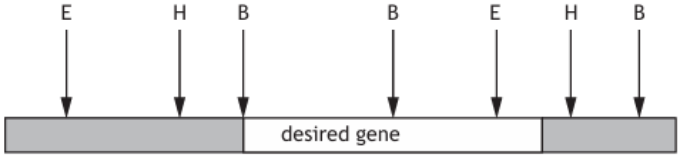
9. The diagram below shows part of a DNA strand and the sites at which three different endonucleases can cut the strand.

- EcoRI endonuclease
- HindIII endonuclease
- SmaI endonuclease

Which line in the table below identifies correctly the number of DNA fragments which would be obtained if this DNA strand was cut with the endonuclease(s) shown in the table?

	Endonucleases			
	EcoRI	*SmaI*	*HindIII*	*EcoRI and HindIII*
A	2	2	1	3
B	2	3	2	3
C	4	2	1	5
D	4	3	2	5

10. DNA recombinant technology can involve the insertion of a desired gene into a plasmid.
The diagram shows restriction sites on a chromosome containing the desired gene.
The restriction sites are H (HindIII), E (EcoR1) and B (BamH1).



The desired gene should be removed with

A HindIII
B EcoR1
C BamH1
D BamH1 and HindIII.

11. Plasmids containing the gene for frost resistance (FRO) and a gene for resistance to the antibiotic ampicillin were incubated with a suitably prepared culture of *E. coli*. The bacteria were then plated out on nutrient agar that either contained ampicillin (+ amp) or did not (– amp).

Which line in the table below shows the status of the bacteria and the agar that would **not** result in colonies?

	Plasmid taken up	*Agar type*
A	Yes	– amp
B	No	+ amp
C	Yes	+ amp
D	No	– amp

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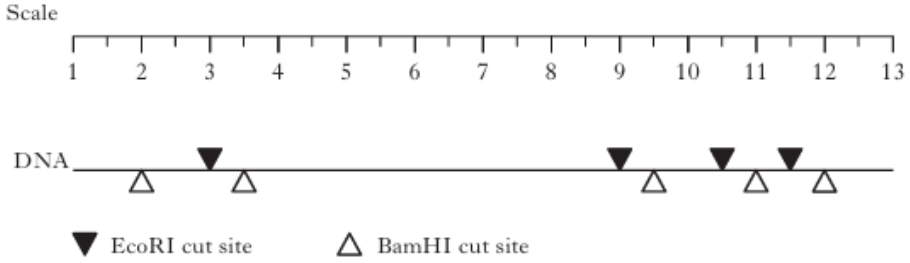
12 In the laboratory, bacteria can be transformed by introducing plasmids that carry genes for new functions. The *transformation efficiency* of the procedure measures how many bacteria take up the plasmid in relation to the mass of DNA used.

$$\text{Transformation efficiency} = \frac{\text{Number of colonies formed}}{\text{Mass of plasmid DNA (µg)}}$$

10 µl of a solution of plasmid DNA at a concentration of 0·08 µg/µl resulted in 480 transformed bacteria. The transformation efficiency is

A 48
B 384
C 600
D 6000.

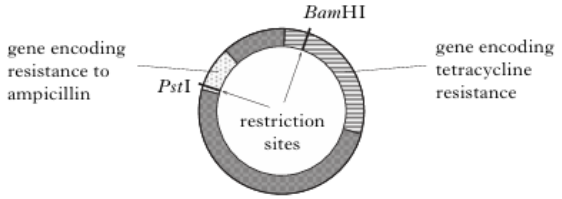
13 A piece of DNA is cut into fragments using the restriction endonucleases BamHI and EcoRI at the sites indicated in the diagram below.



How many bands would be observed following separation of the fragments by electrophoresis?

A 3
B 5
C 6
D 10

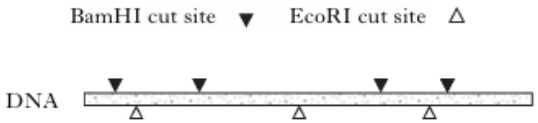
14. The diagram below shows the restriction enzyme sites in a plasmid that carries the genes for resistance to the antibiotics ampicillin and tetracycline.



Which line in the table below identifies correctly the antibiotic resistance that would remain when a gene is inserted at these restriction enzyme sites?

	Gene inserted into restriction enzyme site	*Antibiotic resistance remaining*
A	*Bam*HI	tetracycline and ampicillin
B	*Pst*I	ampicillin
C	*Pst*I	tetracycline and ampicillin
D	*Bam*HI	ampicillin

12. A length of DNA is cut into fragments by the restriction enzymes BamHI and EcoRI.



Which of the following gives the correct number of DNA fragments obtained?

	DNA cut by BamHI only	*DNA cut by EcoRI only*	*DNA cut by both BamHI and EcoRI*
A	5	4	8
B	4	5	8
C	5	4	9
D	4	5	9

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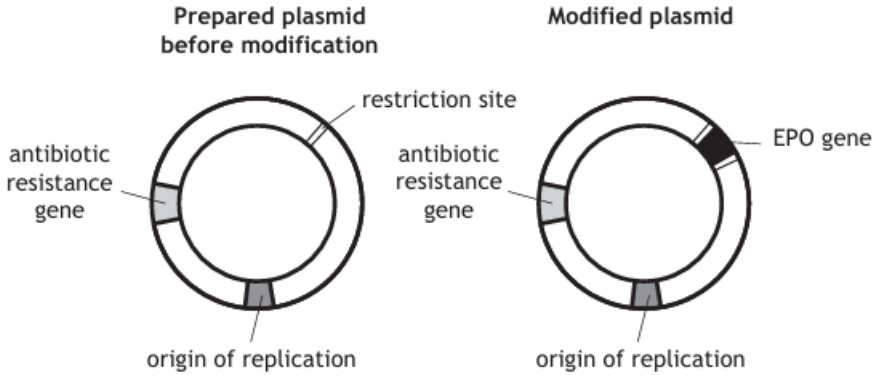
MARKS

1. Erythropoietin (EPO) is a protein synthesised in the kidneys which is involved in red blood cell production. Some individuals with kidney disease have low red blood cell counts and can be treated with EPO.

EPO can be produced by recombinant DNA technology in which the human EPO gene was inserted into a specially prepared bacterial plasmid.

The diagram below shows the prepared bacterial plasmid before and after it was modified by the insertion of a human EPO gene.



(a) Explain the importance of removing the EPO gene from a human chromosome with the **same** restriction endonuclease that was used to open the bacterial plasmid. 1

Complementary sticky ends

(b) Name the enzyme used to seal the EPO gene into the bacterial plasmid. 1

ligase

(c) Modified plasmids were mixed with bacteria. Some bacterial cells were transformed by taking up the modified plasmids but others were not.

Use information from the diagram to suggest how a culture containing only the transformed bacteria was obtained. 1

Expose bacteria to antibiotics and only those that have taken up plasmids will survive

(d) Identify the section of the modified plasmid shown in the diagram which ensured that it could be copied and passed to daughter cells when transformed bacteria divided. 1

ORI

(e) The EPO protein produced by the transformed bacteria is inactive.

(i) Suggest a reason why bacteria produce EPO protein which is inactive. 1

Cannot fold protein correctly

(ii) Suggest how recombinant DNA technology could be used to produce an active form of the EPO protein. 1

Use yeast vectors instead

2. Name a process by which a wild strain of a micro-organism can be improved to increase the yield of a desired product.

Mutagenesis OR Recombinant DNA technology 1

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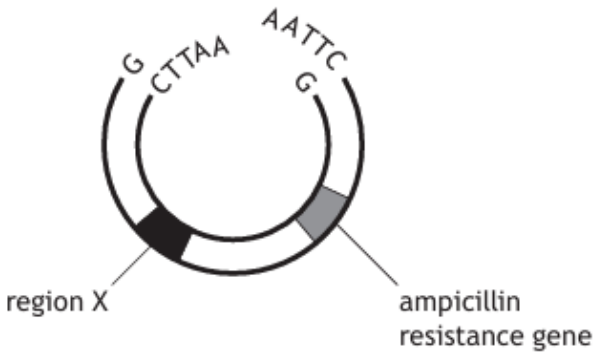
3. The diagram shows some features of a plasmid which has been cut open by restriction endonuclease to allow a gene from a donor chromosome to b inserted.



The DNA recognition sites for three restriction endonucleases, *E coR1*, *BamH* and *HindIII*, are shown in the table. The arrows indicate where each restricti endonuclease cuts the DNA sequence.

Restriction endonuclease	*DNA sequence recognised*
E coR1	G↓AATTC CTTAA↑G
BamH1	G↓GATCC CCTAG↑G
HindIII	A↓AGCTT TTCGA↑A

(a) (i) A restriction endonuclease was used to remove a gene from a donor chromosome.

Use information from the diagram and the table to identify the restriction endonuclease which would be used to allow the gene to be inserted into the plasmid.

Give a reason for your answer. **2**

Restriction endonuclease Eco R1

Reason Complementary sticky ends/ DNA sequence

(ii) Name the enzyme which would be used to seal the gene into the plasmid. **1**

ligase

(iii) A culture of bacterial cells, 20% of which had taken up this modified plasmid, were grown on a nutrient agar plate. The plate was incubated and 250 colonies of this bacteria grew.

Predict the number of colonies which would have been expected to grow if the nutrient agar plate had contained the antibiotic ampicillin. **1**

Space for calculation

50

(b) Name region X, shown in the diagram, which ensured that the modified plasmid would be passed on to daughter cells. **1**

ORI

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4. An investigation was carried out into the effect of increasing time of exposure to UV light on the survival of wild type (WT) and mutant (M) yeast cells.

Each type of yeast cell was grown in separate liquid media at 30 °C for 24 hours, diluted and plated onto separate agar plates. They were then exposed to a UV light source for between 0 and 30 seconds in a darkened room.

The plates were incubated for four days at 20 °C and the number of yeast colonies that had grown was counted. Each colony grew from a single cell.

The results are shown in the table.

Time of exposure to UV light (seconds)	Number of yeast colonies	
	WT	M
0	360	400
5	210	120
10	90	25
15	45	10
20	20	0
30	10	0

(a) (i) State an independent variable in this experiment. 1

Time of exposure to UV light

(ii) Suggest why exposure to UV light was carried out in a darkened room. 1

To prevent other light from affecting the results.

b) Draw **one** conclusion from the results of this investigation. 1

Mutant yeast has lower survival when exposed to UV light.

As length of UV light increases, yeast survival decreases

c) Sunscreen lotions can protect cells from UV damage.

Suggest how the investigation could be modified to test the effectiveness of a sunscreen lotion using M yeast as model cells. 2

Put a layer of sunscreen/ lotion over the plates of

(M) yeast & count colonies produced (1)

Compare with yeast with no sunscreen (1)

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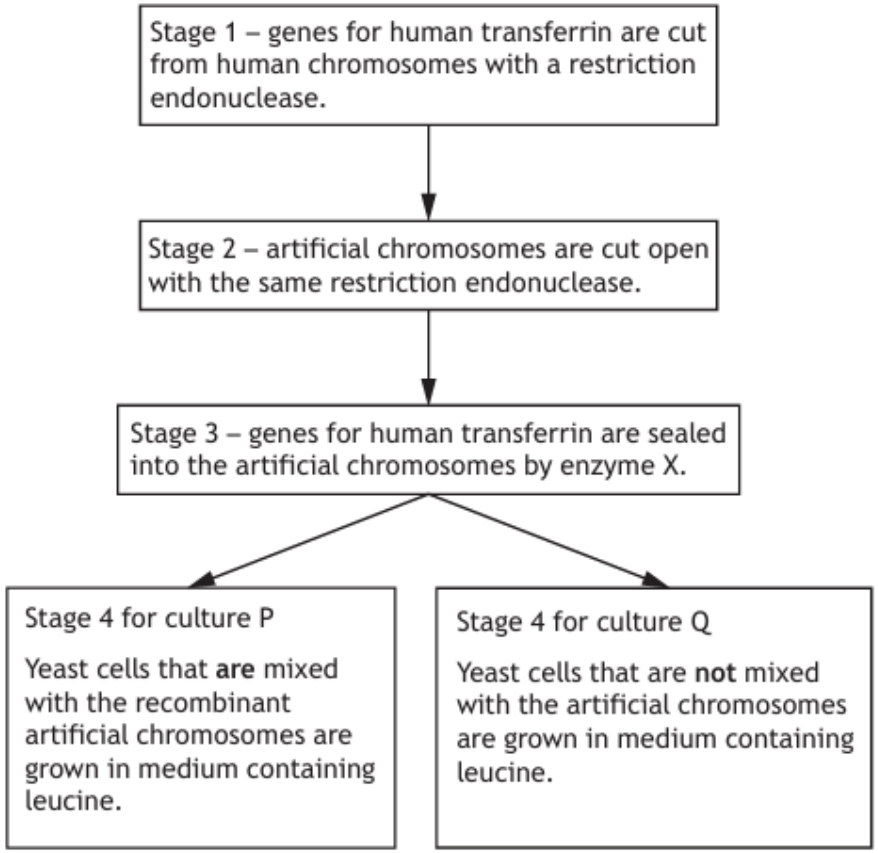
5. Transferrin is a protein used to treat the blood disorder anaemia in humans.

Artificial chromosomes are used in recombinant DNA technology to genetically modify yeast cells to produce transferrin.

The strain of yeast cells used cannot synthesise the amino acid leucine, which is necessary for protein synthesis and growth of the yeast.

Some stages of this recombinant DNA technology process are shown in the diagra



(a) (i) State the term used to describe the artificial chromosome that carries the human gene into yeast cells. **1**

vector

(ii) State why an artificial chromosome rather than a plasmid may be used in recombinant DNA technology. **1**

Carries/transfers larger genes

(b) Explain why the same restriction endonuclease is used in stages 1 and 2. **1**

To ensure complementary sticky ends

(c) Name enzyme X used in stage 3. **1**

ligasae

(d) The artificial chromosome used contains a selectable marker gene that only allows transformed yeast cells to synthesise leucine.

After stage 4, cultures P and Q were transferred to separate plates containing solid medium without leucine.

After incubation, yeast cells only grew on the plate containing culture P.

Explain this result. **2**

Culture P has been transformed/modified/picked up chromosome.(1)

They can synthesise leucine/amino acid to make proteins (1)

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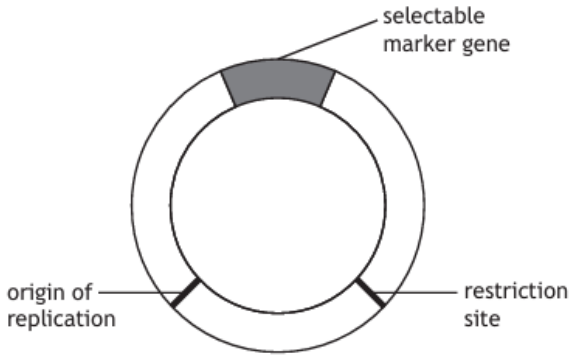
6. Recombinant DNA technology involves the use of plasmids to transfer desired genes into microbial cells.

The diagram shows some key features of a plasmid used in recombinant DNA technology.



(a) (i) State the term used to describe the role of the plasmid in recombinant DNA technology. 1

vector

(ii) Describe the function of the origin of replication. 1

Allows self replication of plasmid

(b) Name the type of enzyme used to remove a desired gene from a chromosome and cut open the plasmid. 1

Restriction endonuclease

(c) When bacteria take up recombinant plasmids they are said to be transform

The selectable marker gene on the plasmid allows transformed bacteria to identified.

Give an example of a selectable marker gene and explain how it allows transformed bacteria to be identified.

Selectable marker gene Antibiotic resistance

Explanation Only transformed bacteria will survive

Antibiotics.

7. *Escherichia coli* is a species of bacteria found in the lower intestines of humans.

Sections of the DNA of two strains of *E. coli* are shown below.

Gene B codes for a protein known as Shiga toxin which can cause serious food poisoning in humans.

Strain of *E. coli*	Section of DNA
0157H7	Gene A | Gene B | Gene C
K12	Gene A | Gene C

(a) The gene for Shiga toxin has been acquired by *E. coli* 0157H7.

Give a method by which the bacterial strain could have acquired this gene.

Horizontal gene transfer 1

(b) (i) *E. coli* K12 is routinely used in recombinant DNA technology. Explain why *E. coli* K12 is used whereas *E. coli* 0157H7 is not.

E. Coli 0157H7 has gene B which produces shiga toxin which can cause food poisoning. 1

(ii) Bacteria are used in recombinant DNA technology.

Explain why animal DNA which has been transferred to bacteria may produce proteins which are not functional.

Bacteria cannot fold protein properly 1

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Mark:

8. **Diagram 1** below shows a plasmid that contains two genes for antibiotic resistance. **Diagram 2** shows the same plasmid after modification by the insertion of a gene from another organism.

Diagram 1
Plasmid before modification

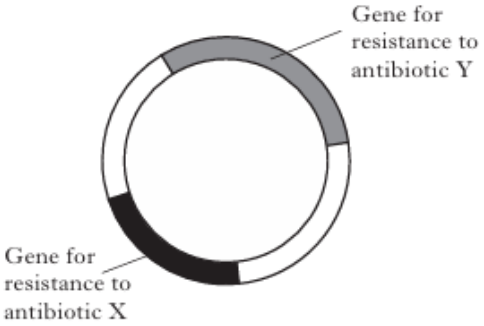
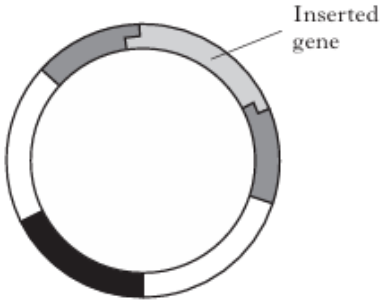


Diagram 2
Plasmid after modification



(*a*) Two different enzymes are required to produce the modified plasmid.

Complete the table below by:

(i) naming the enzyme which cuts plasmids at specific restriction sites; **1**

(ii) naming the second enzyme required to modify plasmids; **1**

(iii) describing the function of the second enzyme. **1**

Enzyme required to produce modified plasmids	*Function of enzyme in the modification process*
Restriction endonuclease	Cuts plasmids at specific restriction sites
Ligase	Seals gene into plasmid

(*b*) Bacteria took up the modified plasmids.

Explain why these bacteria will not have resistance to antibiotic Y.

Antibiotic Y is interrupted so cannot produce protein protectly.

1

9. Write notes on plasmids as vectors in recombinant DNA technology. **4**

1. Vector is a DNA molecule used to carry (foreign) DNA into another cell/genome/organism
2. Restriction site where restriction endonuclease cuts/gene inserted
3. Regulatory sequence that controls gene expression
4. Origin of replication allows plasmid to make copies of itself
5. Selectable marker/antibiotic resistance gene allows only bacteria that have taken up plasmid to grow **1**
6. Ligase seals/inserts the gene (into the plasmid)

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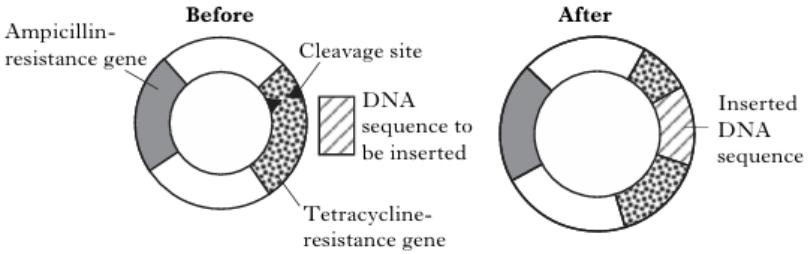
10 Genetic manipulation of bacteria can be achieved by the introduction of plasmids containing DNA sequences from other organisms. The diagram shows a plasmid containing resistance genes for the antibiotics ampicillin and tetracycline, before and after the insertion of a DNA sequence.

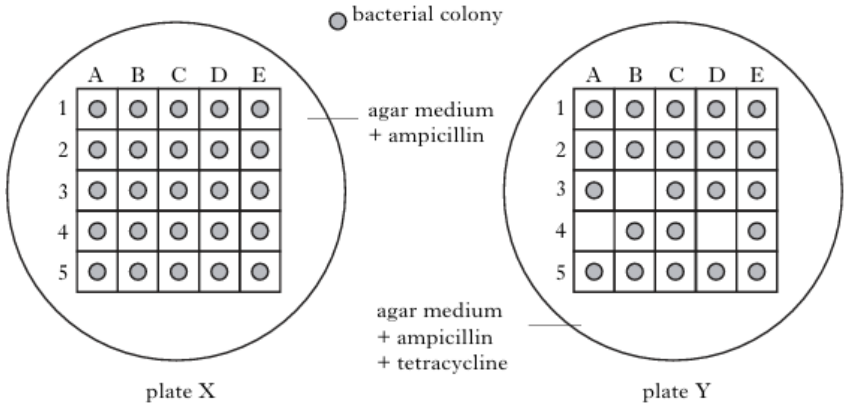


There are two possible problems with this approach:

(i) only some of the plasmids take up the DNA sequence;

(ii) only a small percentage of bacterial cells take up a plasmid of any kind.

Bacteria containing plasmids with the desirable DNA sequence can be selected by culturing them on media containing antibiotics. The same colonies are plated in the same grid positions on the two plates shown below.



(a) What is the effect of inserting the DNA sequence into the tetracycline-resistance gene? **1**

Bacteria does not have resistance to tetracycline

(b) Explain why the colonies A4, B3 and D4 from plate Y would be selected for further analysis. **2**

These colonies contain the modified plasmids (1)

As they grow on plate X/plate with ampicillin but not plate Y/tetracycline (1)

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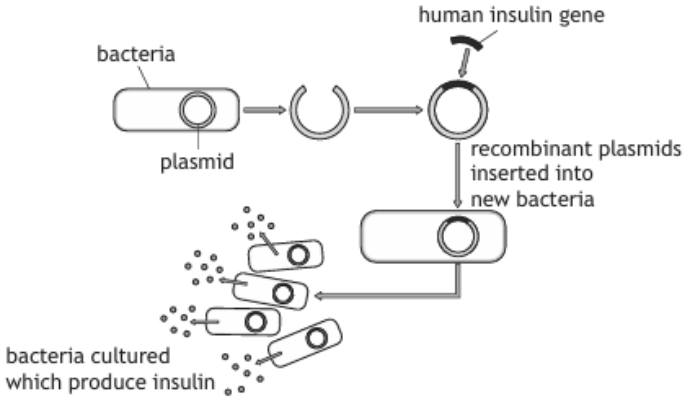
MARKS

11 The diagram below shows how a human gene can be inserted into bacteria to produce human insulin using recombinant DNA technology.



(a) Name **one** enzyme used in this process and state its function. 2

Name Restriction endonuclease OR ligase

Function Cuts plasmid open OR cuts gene out of chromosome OR seals gene into plasmid

(b) (i) The recombinant plasmid also contains a gene for resistance to the antibiotic, ampicillin.

Describe a procedure which would allow only cells containing the recombinant plasmid to be selected. 2

- expose bacteria to antibiotics
- Only those that have taken up plasmid will survive antibiotic

(ii) Plasmids with these antibiotic resistance genes have been passed to other bacterial species by horizontal transfer.

Describe the process of horizontal transfer. 1

Genes are passed between bacteria in the same generation

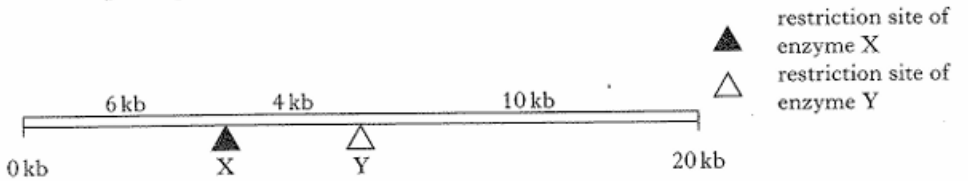
(c) When culturing the bacteria which produce insulin, sterile conditions are maintained.

Explain why this is important. 1

To outcompete other microbes

Marks

12 Restriction enzymes cut strands of DNA double helix at specific sites called *restriction sites*. The effect of using the enzymes is to cut the DNA into fragments, which can be separated by electrophoresis. *Restriction maps* can be created from the fragment information to show the relative positions of the restriction sites in kilobase (kb) lengths, as shown in the following example.



(a) What sizes of fragment would be produced if the 20 kb piece of DNA shown in the map above was cut with enzyme X only? 1

6 & 14

(b) The table shows the number and sizes of fragments from a digest of a 10 kb DNA sample using two restriction enzymes, A and B.

Enzymes added	*Fragment size* (kb)		
A only	8	2	–
B only	7	3	–
A and B together	5	3	2

Draw a restriction map for the digest, labelling where the restriction sites are in relation to each other. 1

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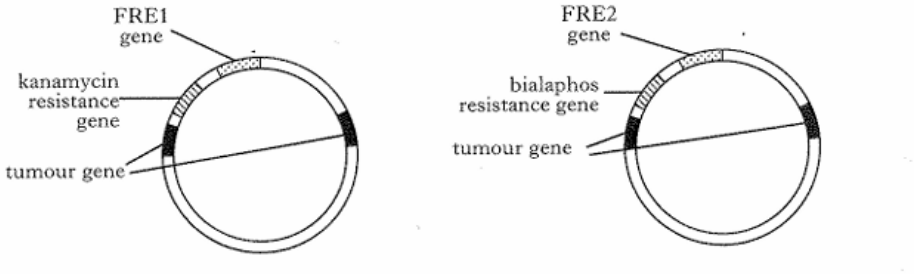
13 Agrobacterium Tumefaciens is a bacteria which can infect plants via its tumour inducing (Ti) plasmids causing tumour like growths at the base of the plant stem.

The aim of the investigation was to determine the effect of iron uptake genes on genetically engineered tobacco plants' growth.

Ti plasmids can be modified to inhibit tumour formation and FRE 1 or FRE 2 genes added which facilitate plant iron uptake from the soil via the plant roots. Iron is an important nutrient for plant growth as it is needed for chlorophyll formation.

Modified Ti plasmids with FRE 1 or 2 genes were incubated with tobacco plant cells in media to produce transformed tobacco plants. To check the success of the transformation process, Ti plasmids had kanamycin or bialaphos resistance genes added and grown in appropriate media.



Two groups of plants were produced to begin with, one with the FRE 1 gene and the other with the FRE 2 gene. These plants were then bred together to produced a third group with both FRE 1 and FRE 2 genes. A control group was also tested at the same test.

The results of the iron concentration of tobacco plant leaf tissues grown in high and low iron soil are shown below.

	Iron concentration (µg/g dry mass)	
Plant Group	*"High" Iron*	*"Low" Iron*
Controls	129	26
KA (FRE1)	154	31
BI (FRE2)	199	43
KA+BI (FRE1+2)	223	41

a) Describe how the control group was set up.

Exact Same set up but plasmids do not contain iron uptake genes.

b) When tobacco cells take up the modified Ti plasmid they are said to be transformed.

For the FRE 2 Ti plasmid state the selectable marker and explain how it allows detection of only transformed tobacco plants.

Selectable marker bialaphos

Explanation Only transformed tobacco plant tissue will survive Media with bialaphos

C) Draw one conclusion from the results of the investigation

Iron uptake genes increase tobacco plant growth