Biology Paper 2 Electives BY TOPIC AL CE DSE 1980-2022	TOPIC
Biotechnology	**Techniques in modern biotechnology**
	Application in biotechnology
	Bioethics

SECTION D Biotechnology

Answer **ALL** parts of the question.

4(a) Sickle cell anemia is an autosomal genetic disorder caused by a recessive mutation in the gene coding for a polypeptide chain of haemoglobin. With this mutation, the seventh amino acid of the polypeptide is changed to another amino acid. Part of the DNA sequence of the mutated allele is shown below:

primer 1 → number of bases

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AGTCAGGGCA GAGCCATCTA TTGCTTACAT TTGCTTCTGA CACAACTGTG  50
TTCACTAGCA ACCTCAAACA GACACCATGG TGCATCTGAC TCCTGTGGAG 100
AAGTCTGCCG TTACTGCCCT GTGGGGCAAG GTGAACGTGG ATGAAGTTGG 150
TGGTGAGGCC CTGGGCAGGT TGGTATCAAG GTTACAAGAC AGGTTTAAGG 200
AGACCAATAG AAACTGGGCA TGTGGAGACA GAGAAGACTC TTGGGTTTCT 250
GATAGGCACT GACTCTCTCT GCCTATTGGT CTATTTTCCC ACCCTTAGGC 300
TGCTGGTGGT CTACCCTTGG ACCCAGAGGT TCTTTGAGTC CTTTGGGGAT 350
CTGTCCACTC CTGATGCTGT TATGGGCAAC CCTAAGGTGA AGGCTCATGG 400
CAAGAAAGTG CTCGGTGCCT TTAGTGATGG CCTGGCTC              438
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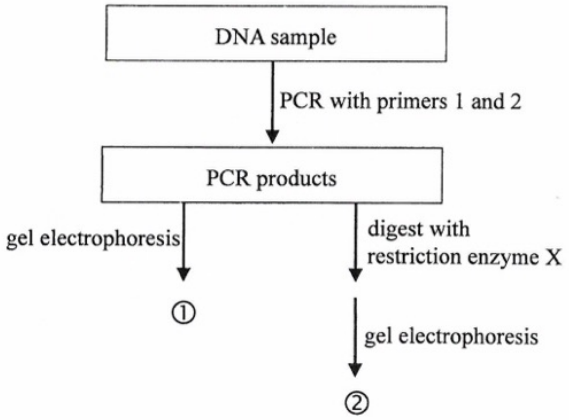
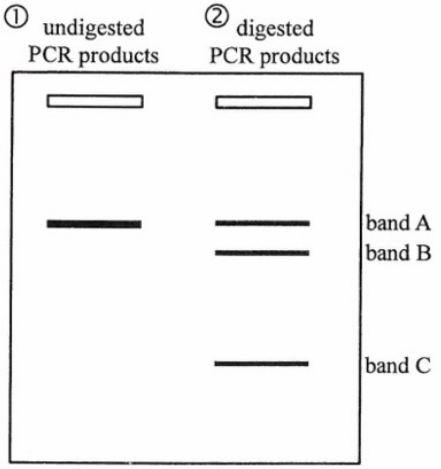
← primer 2

(i) The start of the polypeptide is encoded by genetic code ATG underlined in the above diagram. Write down the genetic code in which the mutation has occurred. (1 mark)

(ii) With reference to the above diagram, what is the expected size of the PCR product when primers 1 and 2 are used to amplify the mutated allele? Give the unit for the size of the PCR product. (2 marks)

(iii) It is known that the recognition site of restriction enzyme X is present in the normal allele but has been destroyed in the mutated allele. A DNA sample was obtained from an individual who is a carrier of sickle cell anemia for the following analysis:





(1) Explain why three DNA bands were detected in the gel electrophoresis of digested PCR products. (3 marks)

(2) Which band(s) correspond(s) to the mutated allele? Explain your answer. (3 marks)

(iv) Based on a DNA sequencing project, the table below shows the frequencies of this mutation in two human populations:

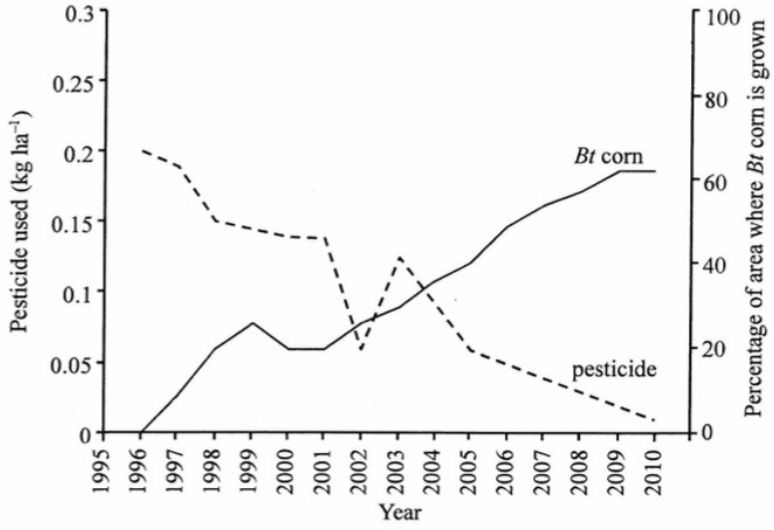
Population	Number of people	Number of mutations	Number of people who are homozygotes
African	12 482	1 121	4
East Asian	9 961	0	0

From which population was the DNA sample in (iii) most likely taken from? (1 mark)

4(b) *Bt* corn is a transgenic crop which contains a *Bt* gene obtained from a certain bacterium. This *Bt* gene can produce a protein which is toxic to certain insect pests.

(i) Agrobacterium transformation is commonly used in the production of transgenic plants. State ***two*** limitations of this method. (2 marks)

(ii) The graphs below show the adoption rate of *Bt* corn and the use of pesticides in the farms of the United States:

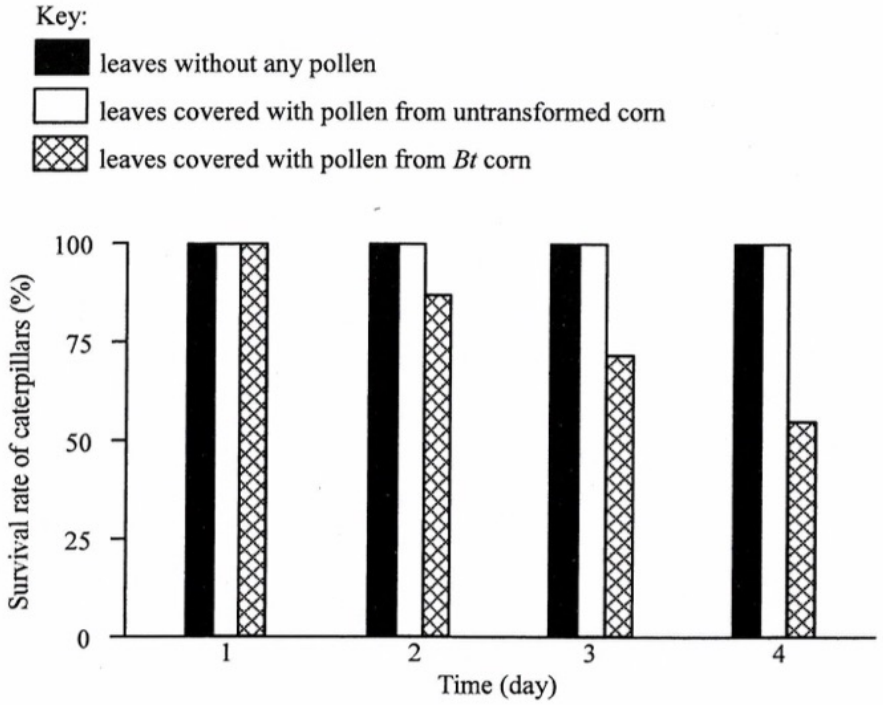


(1) Explain the relationship between the adoption rate of *Bt* corn and the use of pesticides. (3 marks)

(2) State ***one*** advantage for farmers on adopting *Bt* corn. (1 mark)

(iii) A rapid decline in the population of a butterfly species M was noted after *Bt* corn was introduced to the field. *Bt* corn was blamed for the decline.

(1) In a laboratory study, caterpillars of M were divided into three groups, each fed with a different type of leaves. The survival rates of the three groups of caterpillars are shown below:



Based on the results of this study, suggest a possible explanation for the decline of the population of M. (2 marks)

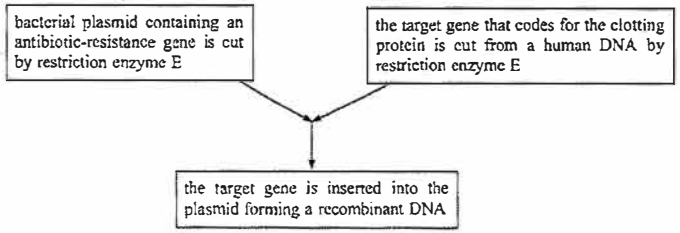
(2) Later, large-scale field studies were conducted by placing caterpillars of M at different distances away from the edge of *Bt* corn fields and untransformed corn fields. It was discovered that there was no significant difference in the survival rates of these caterpillars.

Scientists cast doubt on the validity of the laboratory findings in (1). Suggest ***two*** conditions in the fields which may affect the validity of the laboratory findings. (2 marks)

Biotechnology

Technology in Modern biotechnology
2012sp(4)

4.(a) In some people, a gene mutation results in a failure to produce a blood clotting protein and this leads to the disease haemophilia. One way to treat this disease is by introducing an external source of the clotting protein into these people's blood. The clotting protein can be produced by recombinant DNA technology. The flow chart below outlines the major steps of producing the recombinant DNA:



(i) (1) What is a bacterial plasmid? (1 mark)

(2) Give *two* reasons why plasmids are commonly used as vectors in recombinant DNA technology. (2 marks)

(ii) In most cases, the restriction enzyme used to cut the target gene from the human DNA should be the same as that used for cutting the plasmid. Explain the importance of this in the formation of the recombinant DNA. (2 marks)

(iii) Outline how the clotting protein is produced on a large scale after obtaining the recombinant DNA. (4 marks)

(iv) Traditionally, the clotting protein is obtained by extraction from donated blood. Give *two* advantages of using the clotting proteins produced by recombinant DNA technology over that obtained from the traditional source. (2 marks)

4. (b) Polymerase chain reaction (PCR) is a technique in modern biotechnology. A cycle of PCR consists of three principal steps, which operate at 95°C, 55°C and 72°C in sequence.

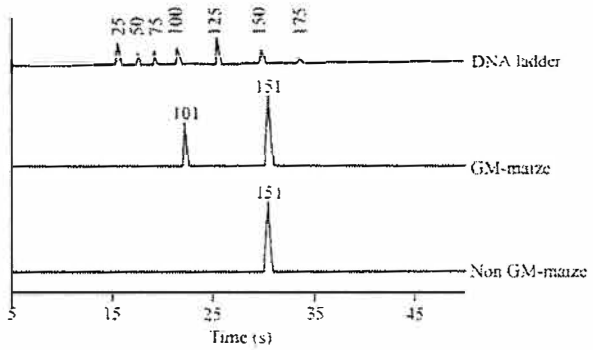
(i) Outline what happens in the three principal steps in a cycle of PCR. (3 marks)

(ii) One application of PCR is Polymerase Chain Reaction–Short Tandem Repeat Analysis (PCR–STR analysis) which can be used in forensics. In a crime scene, a piece of hair suspected to be the criminal's is found. A suspect is arrested one week later.

(1) With reference to the above case, state the significance of PCR in PCR–STR analysis. (1 mark)

(2) Describe how the products of PCR are used in PCR–STR analysis to produce evidence for verifying whether the suspect has committed the crime. (3 marks)

(iii) Another application of PCR is for identifying GM organisms. The following shows the analysis of the DNA of a GM maize and a non-GM maize:
[Note: The number above each peak in the figure indicates the number of base pairs (bp).]



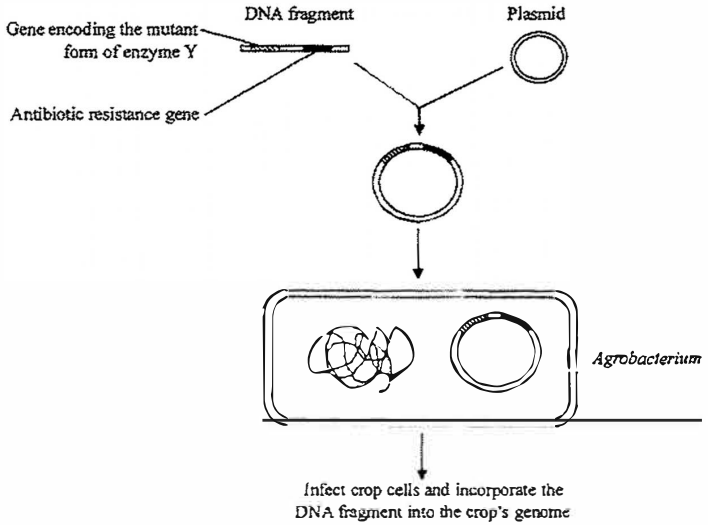
(1) A marker (a polynucleotide chain) with 101 bp is introduced to the maize in producing the GM maize. With reference to the above analysis, suggest the purpose of introducing a marker to the maize in the process of producing the GM maize. (2 marks)

(2) Illustrate with an example how GM plants can help promote people's health. (2 marks)

(3) What are the possible impacts on the ecosystem of growing GM plants that produce a toxin to kill insects? Describe two possible impacts. (2 marks)

4.(a) Chemical X inhibits enzyme Y in photosynthesis and kills all plants. A scientist identified a mutant form of enzyme Y that works properly in photosynthesis and is not inhibited by chemical X. He cloned the gene encoding the mutant form of enzyme Y and put it into a plasmid so that the gene can be transferred into some cells of a crop species. Figure 4A below is a diagrammatic representation of the process:

Figure 4A



(i) Describe *two* steps taken to put the DNA fragment into the plasmid. (2 marks)

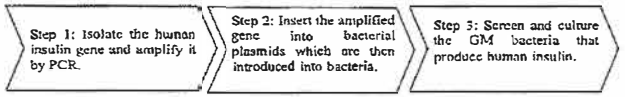
(ii) The scientist then transferred the plasmid into the cells of a crop plant using *Agrobacterium*. After the treatment, only some crop cells have incorporated with the DNA fragment.

(1) Explain why only some of the treated crop cells have incorporated with the DNA fragment. (2 marks)

(2) How can the scientist identify those crop cells that carried the DNA fragment? (3 marks)

(iii) The scientist suggests that growing this genetically modified crop together with chemical X applied to the field can greatly increase the crop yield. Explain the reasoning for this suggestion. (4 marks)

4(a) In the past, diabetic patients were treated with insulin obtained from animal pancreases. With advances in recombinant DNA technology, insulin is now derived from genetically modified (GM) bacteria. Below shows a possible scheme for developing such a GM bacterium:



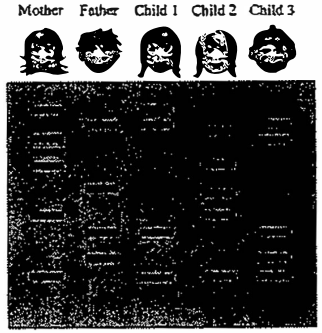
(i) Give *two* raw materials which are necessary in Step 1 for the amplification of the human insulin gene from a DNA template. (2 marks)

(ii) With reference to the immune response, explain why insulin from GM bacteria is used instead of that extracted from animal pancreas. (4 marks)

(iii) Explain why producing insulin from GM bacteria is cheaper than extracting it from animal pancreas. (2 marks)

(iv) To further cut the cost of insulin production, a GM crop which produces human insulin has recently been developed. However, an environmental group is worried that growing such GM crops may cause genetic pollution. Explain why growing the GM crops may cause genetic pollution. (2 marks)

4(b) The diagram below shows the DNA fingerprints of five members of a family, which consists of a couple and three children. One of the children is from the mother's previous marriage.



(i) Based on the information above, deduce which child is from the mother's previous marriage. (3 marks)

(ii) Although the other two children are the biological children of the parents shown, their DNA fingerprints display different patterns. Explain why this is so. (3 marks)

(iii) The different patterns shown in the DNA fingerprinting are due to the presence of variable number tandem repeats (VNTRs) on human chromosomes. VNTRs are short sequences of repeated DNA on the non-coding region of chromosomes and the number of VNTRs varies greatly from person to person. Explain why a large number of variations can exist in VNTRs but fewer variations are found in functional genes. (4 marks)

4(a) In the past, animals with certain desirable traits were selected to breed for several generations and the offspring produced would be domesticated. This technique is known as selective breeding. Nowadays, animals with desirable traits can be produced by animal cloning or transgenic technology.

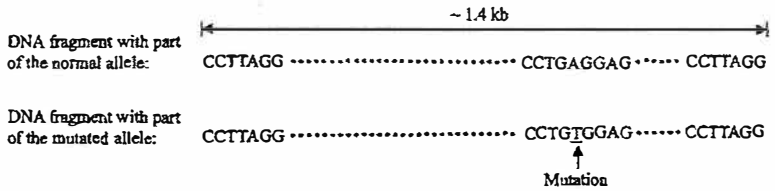
(i) With reference to the principles involved in selective breeding and animal cloning, explain why animal cloning is a better method of preserving the desirable traits of an animal than selective breeding. (5 marks)

(ii) Both selective breeding and transgenic technology affect the gene pool of a species. Transgenic technology is more controversial than selective breeding.

(1) How would selective breeding affect the gene pool of a species? (2 marks)

(2) Production of transgenic organisms is more controversial due to its effect on the gene pool of a species. Explain why. (3 marks)

4(b) DNA fingerprinting is used in the screening of a genetic disease known as sickle-cell anaemia. The disease is a result of a gene mutation which leads to the production of defective haemoglobin. To prepare the DNA fingerprint, copies of DNA fragments containing the gene associated with sickle cell anaemia are first produced by a polymerase chain reaction (PCR). The fragments are then treated by a restriction enzyme which cuts DNA at the middle of CCTNAGG, where N can be any nucleotide. The diagram below shows some nucleotide sequences of the DNA fragment containing the normal allele and the mutated allele for sickle-cell anaemia:



(i) How many restriction sites are found in the DNA fragment with the normal allele and that with the mutated allele respectively? (1 mark)

(ii) Based on the principle of gel electrophoresis, explain how the cutting of the two DNA fragments shown above would produce different DNA fingerprint patterns in a gel. (4 marks)

(iii) How many DNA bands would be observed in the DNA fingerprint of a carrier of sickle-cell anaemia? Explain your answer. (2 marks)

(iv) Explain why the gene mutation will result in the production of defective haemoglobin. (3 marks)

2015(4iii,b)

4(a) Read the following passage and answer the questions that follow it.

'Bubble kid' success puts gene therapy back on track

When Nina was a 5-week-old baby, she had a condition called severe combined immunodeficiency (SCID) which is caused by a faulty *ADA* gene. This gene originally encodes the enzyme adenosine deaminase. The absence of this enzyme will lead to the accumulation of toxins in white blood cells and finally kill the cells. Affected kids have to live in a sterile environment and they are often called 'bubble kids'.

Today, Nina is a happy girl with a functioning immune system. She has gene therapy – and its latest improvements – to thank for this. In the therapy, cells were harvested from Nina's bone marrow and given a working version of the *ADA* gene, before being injected back into her bone marrow. After 5 months, her white blood cell count had nearly doubled, and today her immune system is fully functional.

(i) With reference to the roles of white blood cells, explain why children suffering from SCID have to live in a sterile environment. (2 marks)

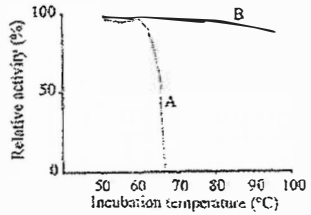
(ii) Why are cells from the bone marrow instead of white blood cells used in the gene therapy for SCID patients? (4 marks)

(iii) With reference to Nina's case, briefly describe how recombinant DNA technology is applied in gene therapy. (3 marks)

(iv) Suggest the potential hazards of gene therapy. (2 marks)

4(b) (i) Briefly describe the three major stages involved in a PCR cycle. (3 marks)

(ii) To test heat resistance of DNA polymerases A and B, the polymerases were incubated at different temperatures for 30 minutes. Their relative enzyme activities as compared to the maximum activity of the enzyme were measured. The results are shown in the graph below:



(1) Of the three stages mentioned in (i), which stage requires the action of DNA polymerase? (1 mark)

(2) Which DNA polymerase is more suitable for use in PCR? Explain your answer. (4 marks)

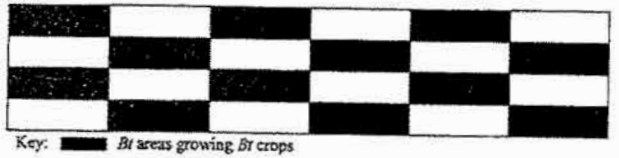
(iii) State *one* application of PCR. (1 mark)

2016(4b (ii, basic genetic in compulsory)

4(b) *Bt* crops were one of the early transgenic plants produced to fight against pests. They contain a *Bt* gene that produces a chemical (*Bt* toxin) which is toxic to the larvae (caterpillars) of the pest.

(i) In the production of *Bt* crops, soil bacteria are used to infect and transform the original crops. Outline the steps that should be taken to modify the soil bacterium in preparation for the infection. (4 marks)

(ii) Many scientists are concerned that the overuse of *Bt* crops will produce pests resistant to the *Bt* toxin. *Bt* resistance is a result of a mutation in the pests. The mutated allele (b) is recessive to the wild type allele (B). After feeding on *Bt* crops, only the homozygous recessive (bb) caterpillars can survive. To minimise the emergence of *Bt* resistance, scientists divided farmland into small areas, some growing *Bt* crops and others growing normal crops, as shown in the diagram below:



Key: *Bt* areas growing *Bt* crops

Non- *Bt* areas growing normal crops

(1) It was found that the mutated allele is very rare in the pest population.

(I) Compare the population size of the adult pests found in the *Bt* areas and non-*Bt* areas. (1 mark)

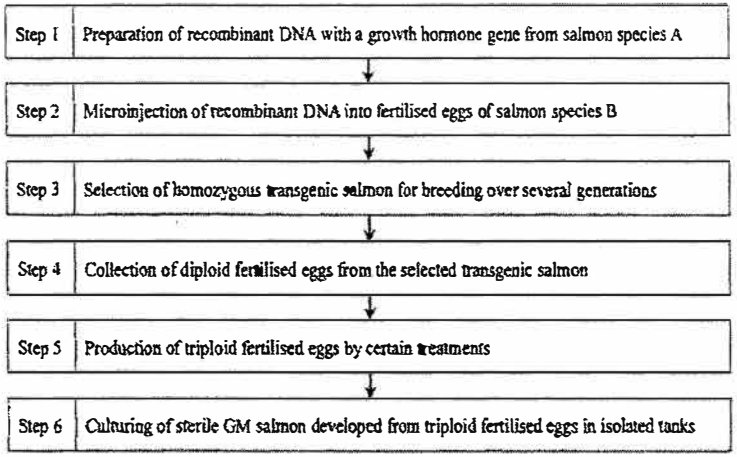
(II) State the genotype(s) of the adult pests found in the *Bt* areas. (1 mark)

(III) State the genotype(s) of the adult pests that make up the largest proportion in the non-*Bt* areas. (1 mark)

(2) (I) Explain how this strategy (growing crops in the above pattern) works in minimising the emergence of *Bt* resistance in the offspring of adult pests. (3 marks)

(II) What is the assumption behind this strategy? (1 mark)

4(a) A genetically modified (GM) salmon was approved for consumption in the United States in 2015. The flowchart below shows some simplified steps involved in the production of the GM salmon:



(i) What is the advantage of culturing this type of GM salmon over non-GM salmon? Explain your answer. (2 marks)

(ii) Microinjection method is used in step 2.

(1) Give *one* advantage of using the microinjection method instead of viral vectors for producing the GM salmon. (1 mark)

(2) Give *one* disadvantage of using the microinjection method. (1 mark)

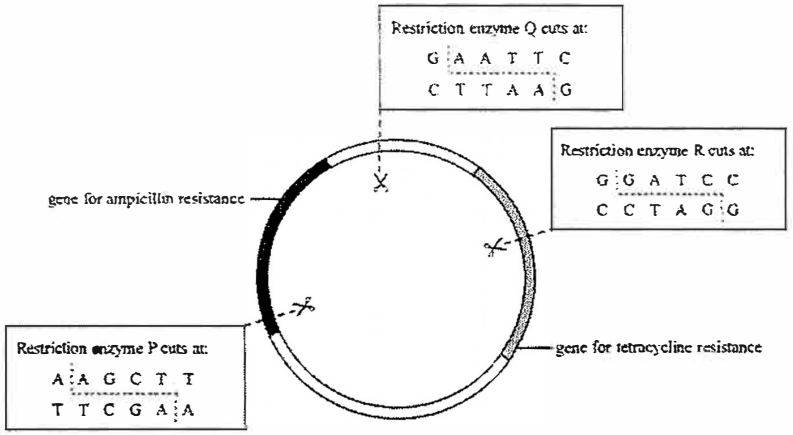
(iii) With reference to step 3, suggest *two* reasons why the selection was made over several generations rather than one generation. (2 marks)

(iv) In step 5, the triploid fertilised eggs produced contain three sets of chromosomes.

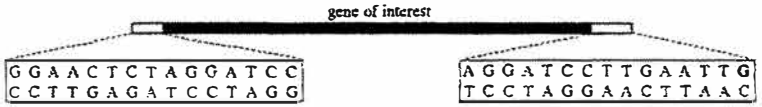
(1) With reference to the process of gamete formation, explain why the GM salmon developed from triploid eggs are sterile. (2 marks)

(2) Explain why this can act as a safety precaution for protection of wild lifes. (2 marks)

4(b) The diagram below shows a plasmid with two antibiotic resistance genes and three cut sites for restriction enzymes P, Q and R:



A student wanted to insert the following DNA fragment into this plasmid which was then used to transform bacteria. Only part of the nucleotide sequence of the two ends of the DNA fragment is shown:



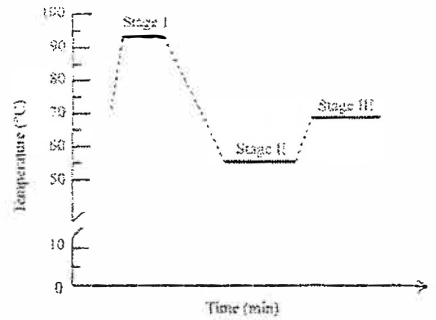
(i) Based on the information above, choose one restriction enzyme to cut both the plasmid and the DNA fragment so that they can be successfully recombined together. Explain your answer. (4 marks)

(ii) After transformation, the bacteria were transferred to an agar plate containing ampicillin for selection. Explain the importance of this step. (3 marks)

(iii) For the bacterial colonies formed on the agar plate containing ampicillin, some of them have tetracycline resistance while some do not. Explain this phenomenon. (3 marks)

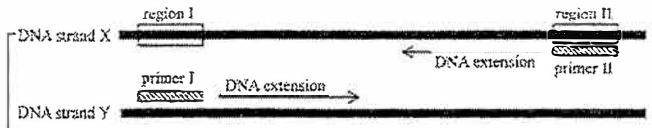
2018(4b)

4(b) The diagram below shows the change in temperature during a polymerase chain reaction (PCR) cycle.



(i) Which stage corresponds to DNA denaturation? Explain your answer. (3 marks)

(ii) Mary planned to amplify a fragment of DNA using PCR. The following diagram shows the annealing of primers during PCR. The sequence of DNA strand X is shown below and the corresponding sequences of regions I and II are highlighted:



Sequence of DNA strand X:

					no. of bases
TGGCGCTGGG	CGCAATGCGC	GCCATTACCG	AGTCCGGGCT	GCGCGTTGGT	50
GCGGATATCT	CGGTAGTGGG	ATACGACGAT	ACCGAAGACA	GCTCATGTTA	100
TATCCCGCCG	TTAACCACCA	TCAAACAGGA	TTTTCGCCTG	CTGGGGCAAA	150
CCAGCGTGGA	CCGCTTGCTG	CAACTCTCTC	AGGGCCAGGC	GGTGAAGGGC	200
AATCAGCTGT	TGCCCGTCTC	ACTGGTGAAA	AGAAAACCA	CCCTGGCGCC	250
CAATACGCAA	ACCGCCTCTC	CCCGCGCGTT	GGCCGATTCA	TTAATGCAGC	300
TGGCACGACA	GGTTTCCCGA	CTGGAAAGCG	GGCAGTGAGC	GCAACGCAAT	350
TAATGTGAGT	TAGCTCACTC	ATTAGGCACC	CCAGGCTTTA	CACTTTATGC	400
TTCGGCTCG	TATGTTGTGT	GGAATTGTGA	GCGGATAACA	ATTTCACACA	450

region I

region II

Mary designed the following primers for the PCR:

Primer I: CGGUAGUGGG AUACGACGAU DNA extension →

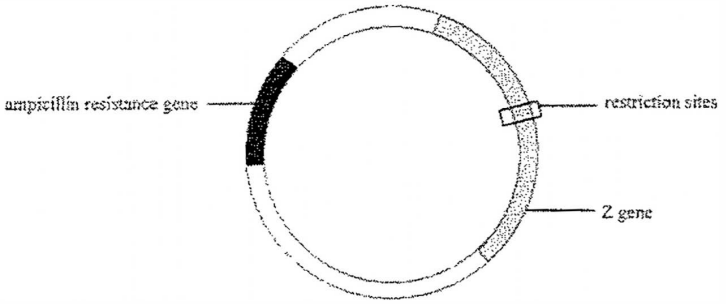
Primer II: CCTTAACACT CGCCTATTGT DNA extension →

(1) There is one type of mistake in each primer. Write the correct primers to be used. (2 marks)

(2) What is the predicted size (in terms of number of base pairs) of the PCR product? (1 mark)

(iii) Mary used the following plasmid as a vector to carry the PCR product to transform bacteria. The plasmid contained:

(I) an ampicillin resistance gene;
(II) a Z gene encoding an enzyme that converts substance X to blue compounds;
(III) some restriction sites within the Z gene.

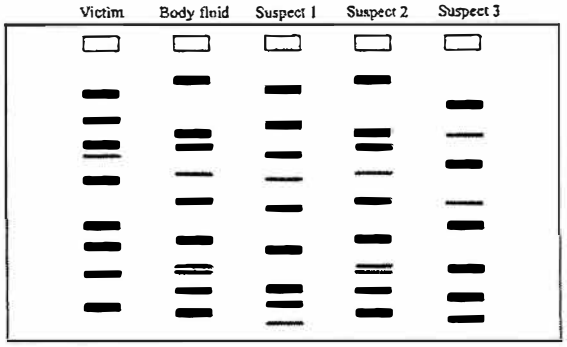


After the transformation of the bacteria, Mary grew the bacteria on agar plates containing both ampicillin and substance X. Blue and white bacterial colonies were formed.

(1) What is the purpose of adding ampicillin to the agar plates? Explain your answer. (2 marks)

(2) Explain which type of colony (blue or white) contains non-recombinant plasmids, i.e. without DNA insert. (4 marks)

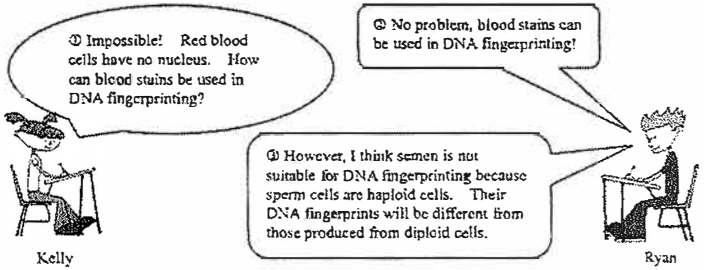
4(a) Samples of body fluids or cells collected from crime scenes can be examined with VNTR-based DNA fingerprinting. The diagram below shows the DNA fingerprints of body fluid samples collected from a crime scene, and those of the victim and three suspects:



(i) Based on the above results, deduce who is most likely the criminal among the three suspects. (1 mark)

(ii) Explain why the DNA fingerprints of these suspects showed different patterns. (3 marks)

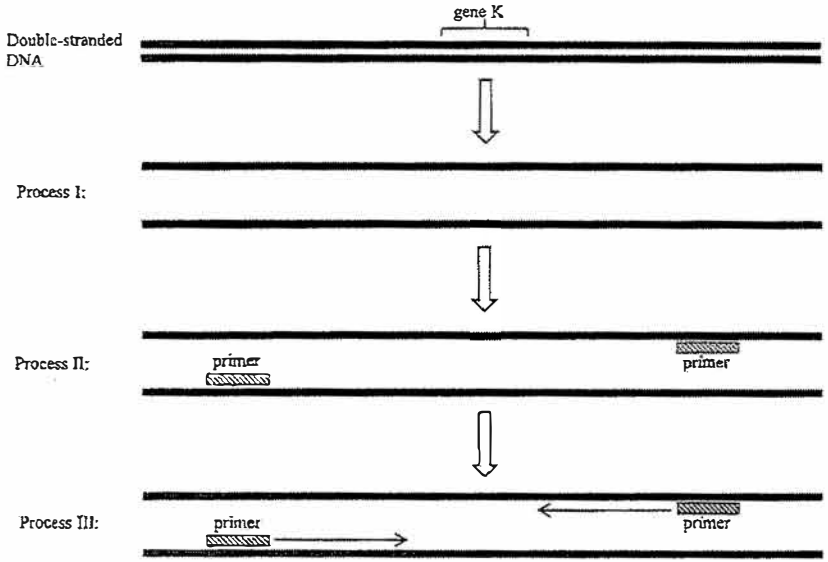
(iii) Semen and blood stains are body fluid samples which can be collected from crime scenes. Kelly and Ryan disagree about the suitability of using these body fluid samples for DNA fingerprinting. Below is their conversation:



(1) Is Kelly or Ryan right about whether blood stains can be used for DNA fingerprinting? Explain your answer. (2 marks)

(2) Do you agree with Ryan's comment about the suitability of using semen for DNA fingerprinting? Explain your answer. (4 marks)

4(b) A scientist discovered a bacterial species capable of secreting a protein. This protein is toxic to insects that damage the roots of a certain crop. The protein is encoded by gene K. The scientist used polymerase chain reaction (PCR) to amplify gene K. The diagram below shows some PCR processes:



(i) What is Process II? (1 mark)

(ii) Draw a simple labelled diagram to show the expected PCR product. (2 marks)

(iii) Primers often contain about 18–22 bases. If primers with fewer bases are used, PCR products of different sizes are obtained. Explain this phenomenon. (2 marks)

(iv) The scientist wanted to transfer gene K into the crop by using *Agrobacterium* (a soil bacterium). Explain why *Agrobacterium* is suitable for transferring the target gene into the crop. (2 marks)

(v) The cultivation of this transgenic crop was found to produce higher yields than its non-transgenic counterpart. Suggest an explanation for this. (3 marks)

4(a) Study the diagrams below. Diagram X shows a DNA segment with a target gene. Diagram Y shows a plasmid with a size of 4 300 base pairs (bp). The restriction sites for restriction enzymes EcoRI, PstI and HindIII are shown in both diagrams.

Diagram X

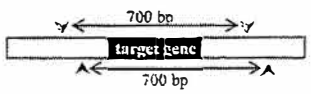
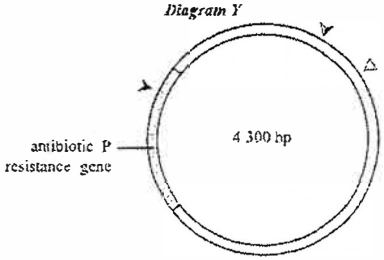


Diagram Y



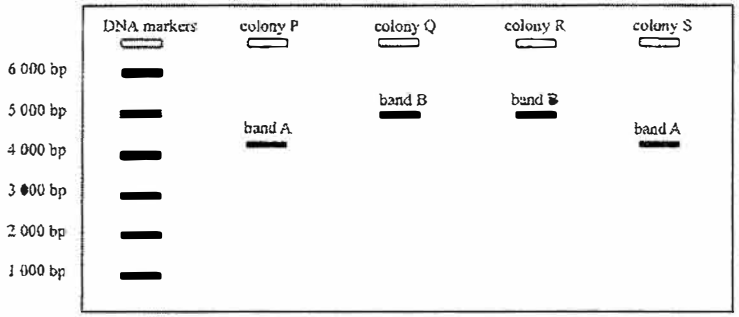
Key: restriction site for EcoRI
restriction site for PstI
restriction site for HindIII

(i) A student plans to insert the target gene into the plasmid and screen the transformed bacteria with antibiotic P.

(1) Which restriction enzyme should be used for the insertion? Explain your answer. (3 marks)

(2) State another type of enzyme which is required to complete the insertion. (1 mark)

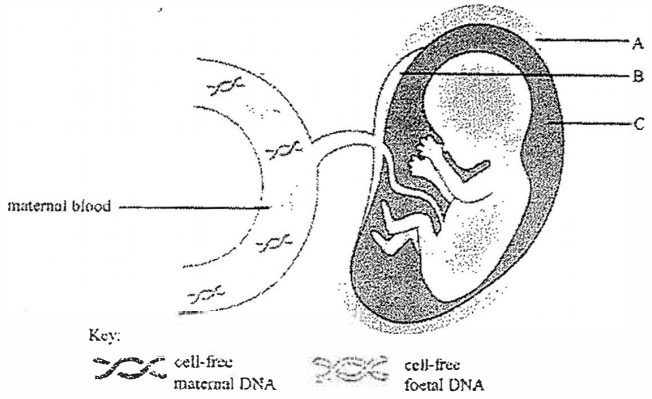
(ii) After transformation, screening and culture, the plasmids from different colonies were harvested and cut using enzyme HindIII. Gel electrophoresis was then conducted to check the size of the cut plasmids. The diagram below shows two types of DNA band obtained:



(1) Which band (A or B) represents the plasmids with the target gene? Explain your answer. (3 marks)

(2) Explain why two types of DNA band were detected in the cut plasmids after gel electrophoresis. (3 marks)

4(b) Traditional diagnosis of Down Syndrome requires the collection of foetal tissue. The recent discovery of cell-free foetal DNA in maternal blood has opened up new possibilities for diagnosis. Cell-free DNA are fragments of DNA released into the plasma from the degradation of normal cells. The diagram below shows a foetus and its associated structures inside the mother's body:



(i) With reference to the above diagram, which labelled structure does the cell-free foetal DNA come from? Explain your answer. (2 marks)

(ii) In the maternal plasma, cell-free foetal DNA is only a minor proportion of the total cell-free DNA. Suggest a technique for enhancing the detection of very small amounts of cell-free foetal DNA in maternal plasma. Explain your answer. (2 marks)

(iii) Nowadays, the identity of the genes in the cell-free DNA can be found through DNA sequencing and mapping against human genome databases.

(1) Scientists proposed that Down Syndrome could be diagnosed by comparing the frequency of occurrence of the genes found on chromosome 21 and another autosome in the cell-free foetal DNA. Based on your understanding about Down Syndrome, explain the biological principle involved in this method. What would be the expected results? (2 marks)

(2) Suggest *two* other possible applications of the results obtained from DNA sequencing. Explain the principle of each application. (4 marks)

bioethics
2012 (4b)(ii)

4.(b) A 25-year-old athlete, Keith, had nerve damage and became paralyzed. In view of the difficulties associated with tissue transplants, he proposed cloning himself, using the method used for Dolly the sheep, so as to get a nerve for transplantation. He discussed his idea with a scientist.

(i) Give *two* problems associated with conventional tissue transplants. (2 marks)

(ii) Keith's idea could not be put into practice. State *one* ethical reason to explain why not. (1 mark)

(iii) The scientist suggested that stem cell therapy may be a way of obtaining some nerve cells.

(1) State *one* place in Keith's body where stem cells can be obtained. (1 mark)

(2) Describe how stem cells may be used to cure Keith. (3 marks)

(3) Describe *two* limitations of using stem cell therapy in this case. (2 marks)

4.(a) (i) (1)
- a plasmid is a small circular piece of extrachromosomal DNA in bacterial cells (1) (1)

(2)
- plasmids can be taken up by bacterial cells because of their small size (1)
- plasmids contain selective markers e.g. antibiotic resistance gene(s) which allow screening for the transformed bacterial cells (1) (2)

(ii)
- the same restriction enzyme would produce the same sticky ends in the target human gene and in the plasmid (1)
- so that the cleaved plasmid and the target gene can be ligated together by complementary base pairing between the unpaired bases in their sticky ends (1) (2)

(iii)
- introduce the recombinant plasmids into some bacterial cells / *E. coli* (1)
- select the transformed bacteria by culturing the bacteria on nutrient agar containing a specific antibiotic (1)
- mass culture the transformed bacterial cells (1) in a nutrient medium containing the antibiotic
- induce the expression of the gene that encodes the clotting protein (1)
- extract and purify the clotting proteins produced (1) (max. 4)

(iv) Any two of the following: (2)
- larger quantities of the clotting proteins can be produced (1)
- lower risk of infection by blood borne diseases (1)
- less side effects due to higher purity of the clotting proteins (1)

Total: 11 marks

4.(b) (i)
- extract DNA sample from the individual (1)
- use restriction enzyme to cut the sample into fragments (1)
- separate the fragments of different sizes using electrophoresis (1)
- match the DNA fingerprint patterns between individuals under study (1) (4)

(accept other correct alternatives)

(ii) (1)
- P is the father / parent of R (1)
- the bands of R are either common to those of P or those of Q (1), indicating that P and Q are R's parents (2)

(2)
- S (1)
- there are bands in his DNA fingerprint that are found neither in the DNA fingerprint of P nor that of Q (1) (2)

(iii) Any one of the following: (accept other correct alternatives) (1)
- as a forensic tool for identifying suspects / victims / human remains / missing persons (1)
- applications in palaeontology (1)
- applications in archaeology (1) (e.g. matching fragments of the Dead Sea Scrolls)
- applications in medical diagnostics (1) (screening patients and foetuses for inherited diseases)
- matching organ donors
- establishing the provenance / composition of foods
- analysing patterns of human migration (1)
- analysing claims of ethnicity (1)

Total: 9 marks

PP P2 4b

4.(b) (i) At 95°C, the complementary stands of DNA are separated into two single DNA strands. 1
At 55°C, primers are annealed to the single stranded DNA templates by complementary base pairing. 1
At 72°C, DNA polymerase adds nucleotides to (the 3' end of) the primers to synthesise the new DNA. 1

(ii) (1) PCR amplifies the small amount of DNA present in the hair follicle for further analysis. 1

(2) The products of PCR are separated by gel electrophoresis into bands according to their molecular masses / sizes. 1
The banding pattern produced after gel electrophoresis is the 'DNA fingerprint' of the owner of the piece of hair. 1
The DNA fingerprint is then compared to that of the suspect to see if they match. 1
Positive matching can be used as evidence against the suspect as the DNA of an individual is unique.

(iii) (1) As shown in the analysis, the marker in the GM maize will always show up in a DNA analysis. 1
This allows maize cells which have been successfully incorporated the desired foreign gene (transformed maize cells) to be screened for use. 1

(2) Example: GM rice that contains a precursor of vitamin A (*β*-carotene) 1
The incidence of night blindness among people living in poor countries can be reduced if they are consuming this GM rice. 1
(Accept other correct alternatives.)

(3) The GM plants may dispense away from the farmland. They may out-compete other native plants and upset the ecological balance. 1
The toxin may accumulate along the food chain and cause poisoning in consumers in the higher trophic levels. 1
(Accept other correct alternatives.)

2012 P2 4a

4. (a) (i)
- cut the DNA containing the gene encoding enzyme Y and plasmid with the same restriction enzyme to produce compatible sticky ends (1) (1)
- join the enzyme gene and plasmid together using DNA ligase (1) (1)

(ii) (1) Any *two* of the following:
- in constructing the plasmid, some cut plasmids joined by itself to restore the original form without picking up the DNA fragment (1)
- in transferring the plasmid to plant cells, some bacteria did not pick up any plasmid at all (1) (max. 2)
- the bacteria did not infect some of the crop cells (1)

(2)
- grow all the plant cells on an agar plate with the antibiotic (1) (1)
- only those plant cells that has picked up the functional plasmid can survive (1) (1)
- as they contain the plasmid with the antibiotic resistance gene (1) (1)

OR
- cut the DNA obtained from the plant cells with restriction enzymes (1) (1)
- amplify the DNA fragment using PCR (1) (1)
- run a DNA electrophoresis to check for the presence of the DNA fragment which has been inserted into the plasmids (1) (1)

(iii)
- when applying chemical X, plants that are not genetically modified / do not carry the gene encoding the mutant form of enzyme Y cannot survive (1) (1)
- on the other hand, the GM crops can survive (1) (1)
- because these GM crops produce another enzyme that will not be inhibited by chemical X, as a result, they can carry out photosynthesis and survive (1) (1)
- without competition, the GM crops have more resources for growth (1) thus the crop yield can be increased (1)

2013 P2 4

4. (a) (i)
- primers (1)
- deoxyribonucleoside triphosphates (dNTPs) (1) (2)

(ii)
- insulin produced from GM bacteria has the same amino acid sequences as the insulin produced by our body (1)
- so that the patient's immune system does not normally produce antibodies against the insulin after injection / rejection on the insulin occurs (1)
- whereas the amino acid sequence of animal insulin is slightly different from that of human insulin (1)
- thus some patients' immune systems produce antibodies against it to degrade / inactivate / lower the effect of insulin (1) (4)

(iii)
- due to the high growth rate of bacteria, the product yield from GM bacteria is much higher than that from animal pancreas because it takes long time to rear animals (1)
- insulin can be produced continuously from the bacterial culture whereas each animal can provide only a limited amount of animal pancreas (1)
- the cost of purification of insulin from bacterial culture is lower than that from the animal pancreas (1) as it is less complicated (max. 2)

(iv)
- GM plants are often grown out in the open areas (1)
- thus pollen grains from GM plants may pollinate other non-GM crops and are spread to other plants (1) (2)

(b) (i)
- child 2 (1)
- because some of the bands resembles the pattern of the mother (1)
- while the other bands cannot be found in the pattern of the father (1) (3)

(ii)
- they are resulted from fusion of different gametes / random fertilization (1)
- due to independent assortment / crossing over of chromosomes (1)
- each gamete bears different copies of chromosomes from the parents (1) (3)

(iii)
- as VNTRs are located in the non-coding region of chromosomes, any mutations do not affect the survival of the organisms (1)
- and the mutations can pass on to the next generation (1)
- mutations in functional genes, however, may lead to expression of non-functional proteins / failure of expression of these genes (1)
- which may affect the survival of the organisms (1) (4)

therefore, variations of VNTRs pass on from generation to generation leading to huge variations

2014 P2 4 (a→basic genetic, b→molecular genetics in compulsory part)

4. (a) (i)
- selective breeding involves sexual reproduction / fusion of gametes (1)
- which produces offspring with genetic variations (1)
- as a result, the desirable traits may be diluted (1) / desirable traits may not appear / undesirable traits may appear
- however, the organisms produced from cloning are developed from mitosis of the cells from desired donor (1)
- the organisms produced are genetically identical to the donors (1), so all the desirable traits will be preserved (5)

(ii) (1)
- as only individuals with desirable traits were selected for breeding while others were not perpetuated (1),
- the genes for the undesirable traits will be eliminated from the gene pool eventually (1) / the gene frequency of the desirable traits will increase / undesirable traits will decrease (2)

(2)
- the desirable genes may be taken from a different species (1) / may not naturally occur in the organisms to be transformed
- as a result, new genes will be added to the gene pool of a species (1)
- this may produce superior species and pose threats to other species / the long term effect is not yet known / this may create new species artificially (1) (3)

(b) (i)
- No. of restriction sites in the DNA fragment with normal allele: 3
  No. of restriction sites in the DNA fragment with mutated allele: 2 (1)

(ii)
- after cutting with the restriction enzymes, two short DNA fragments will be produced from the DNA fragment with normal allele (1)
- and one long DNA fragments will be produced from the DNA fragment with the mutated allele (1)
- as the DNA fragments will migrate to the (positive) pole in gel electrophoresis (1)
- DNA fragments with shorter lengths will migrate faster than those with longer length (1), forming separate bands on the gel (4)

(iii)
- three DNA bands (1)
- as the person has both the normal allele and the mutated allele (1) / is a heterozygote (2)

(iv)
- change in a nucleotide in the base sequence may lead to a change in the triplet code (1)
- thus may change the amino acid sequence of the protein produced (1)
- as a result, the protein produced may fold to a different shape (1) and lose its function (3)

2015 P2 4a iii

(iii)
- a vector is used to carry the working ADA gene (1)
- it delivers and inserts a working ADA gene / normal gene into Nina's genome / cell (1)
- this inserted gene is expressed to produce a functional protein / enzyme (1), which should overcome the defect

(3)

2015 P2 4b

(b) (i)
- DNA molecules are denatured / separated / unwounded to form single strands at the DNA denaturation stage (1)
- primer with complementary bases anneals to the single-stranded DNA molecule at primer annealing stage (1)
- complementary free nucleotides (dNTPs in PCR) join to the primer accordingly to extend the DNA molecule at the extension stage (1)

(3)

(ii) (1)
- extension stage (1)

(1)

(2)
- as all enzymes and substrate are added at the very beginning, enzymes used in PCR cycle should be able to withstand high temperature (~90°C) during the breaking of DNA molecules to single strand (1)
- the results show that DNA polymerase B still has a high relative activity even after incubation at high temperature (1)
- while DNA polymerase A loses most of its activity (1)
- therefore DNA polymerase B is more suitable (1)

(4)

(iii)
- amplify the DNA for genetic testing such as parentage / forensic application / detection of specific gene sequence (e.g. genes of genetic diseases, genetic markers of GM food) (1)

(1)

2016 P2 4b (ii→basic genetic in compulsory)

(b) (i)
- isolate the *Bt* genes from the source using a restriction enzyme (1)
- use the same restriction enzyme to cut the plasmid for insertion of *Bt* gene (1)
- join the *Bt* gene and the cut plasmid using DNA ligase (1)
- transfer the recombinant plasmid into a soil bacterium (1)

(4)

(ii) (1) (I)
- pollution of adult pests in non-*Bt* areas is much greater than that in the *Bt* areas (1)

(1)

(II)
- *Bt* areas: homozygous recessive (1)

(1)

(III)
- largest proportion in non-*Bt* areas: homozygous dominant (1)

(1)

(2) (I)
- it is likely that the surviving bb adult pests (few) from *Bt* areas will mate with those BB pests (large number) from non-*Bt* areas (1)
- as a result, the offspring produced will be of heterozygous Bb (1)
- which are susceptible to *Bt* toxin / sensitive to *Bt* toxin (1)

(3)

(II)
- there is no mating between the adult pest from *Bt* areas (1)

(1)

2017 P2 4 (a iv→Cell cycle)

4. (a) (i)
- The GM salmon have an additional copy of gene for producing growth hormone (1)
- therefore they should have a faster growth rate / can grow to a larger size in a short time (1) (2)

(ii) (1)
- the recombinant DNA does not contain any viral materials which may cause undesirable effects / immune response on humans / which may regain the ability to cause disease (1) (1)

(2)
- the insertion of the recombinant DNA into the genome of fertilized egg has high failure rate / can cause damage of the fertilized egg / time consuming as only one fertilized egg can be targeted at one time (1) (1)

(iii) Any *two* of the following:
- to ensure that the transgene is inheritable over generations (1)
- and the gene can still be expressed / exert its effect after generations of inheritance (1)
- to produce salmons which are pure bred / homozygous for the transgene (1) (2)

(iv) (1)
- the three sets of homologous chromosomes fail to pair up (1) during meiotic cell division for gamete formation
- so low number of viable gametes / no gametes can be formed (1) (2)

(2)
- to ensure that the GM salmon cannot breed with wild salmon (1)
- to avoid passage of the transgene to others even if they escape to the wild (1) (2)

(b) (i)
- use restriction enzyme R to cut both the plasmid and DNA fragment (1)
- since restriction / cut site of R can be found at both ends of the DNA fragment (1)
- as a result, two sticky ends / single-stranded DNA ends will be produced at the plasmid and on the DNA fragment (1)
- which are complementary (1) for insertion of the DNA fragments into the plasmid (4)

(ii)
- it can be used to select bacteria that contain the plasmid / has been transformed (1)
- because bacteria that have successfully picked up the plasmids will possess gene for ampicillin resistance (1)
- thus they can survive on the culture plate containing ampicillin (1)

OR
- it can be used to eliminate bacteria that do not contain the plasmid / have not been transformed (1)
- because bacteria that have not picked up the plasmids will not possess gene for ampicillin resistance (1)
- thus they are killed on the culture plate containing ampicillin (1) (3)

(iii)
- after insertion of the DNA fragment, the tetracycline resistance gene has been interrupted / is no longer functional / cannot be expressed (1)
- therefore, bacteria that have picked up the plasmids with successful insertion do not have tetracycline resistance (1)
- while bacteria that have picked up self-ligated plasmid / plasmid which has not been cut / plasmid without insertion still possess tetracycline resistance (1) (3)

2018 P2 4b

(b) (i)
- Stage 1 (1)
- the temperature is high enough (1)
- to break the hydrogen bonds between the two strands of double helix DNA (1) (3)

(ii) (1)
- Primer I: CGGTAGTGGG ATACGACGAT (1)
- Primer II: TGTTATCCGC TCACAATTCC (1) (2)

(2)
- 380 base pairs (1) (1)

(iii) (1)
- to screen bacteria which have picked up plasmids during transformation (1)
- as theses bacteria have the plasmid with ampicillin resistant gene, they can survive on agar plates with ampicillin (1)

OR
- to eliminate bacteria that do not have the plasmid / are not transformed (1)
- as they do not have the plasmid with ampicillin resistant gene, they are killed by the ampicillin in the agar plate (1) (2)

(2)
- blue colonies (1)
- for bacteria which have picked up the plasmid without DNA insert, the Z gene remains intact (1)
- as a result, these bacteria / plasmids produce the enzyme (1)
- which convert substance X to blue compounds (1), resulting in blue colonies (4)

4. (a) (i)
- suspect 2 whose DNA fingerprint shares the same pattern as the body fluid's (1) (1)

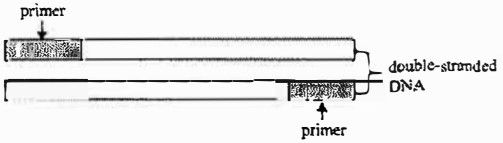
(ii)
- as the number of times the sequence repeats is highly variable / polymorphic / different among individuals (1)
- and different number of repeats results in different lengths of DNA fragments after cutting with suitable enzymes (1)
- since different lengths of DNA fragments migrate with different speeds in gel electrophoresis (1)
therefore, different bands of DNA fingerprinting which is unique to individuals can be shown (3)

(iii) (1)
- Ryan's idea is right (1)
- because blood stains contain white blood cells which have nucleus / has other nucleated cells (1) (2)

(2)
- Ryan's idea is wrong (1)
- although one sperm cell only contains one set of chromosomes (1)
- there are many sperm cells in semen, and each receives chromosomes randomly through meiosis from their diploid mother cells (1)
- thus, the sperm cell lysate formed a collection of chromosomes containing both sets of chromosomes from the diploid mother cells (1) (4)

(b) (i)
- primer annealing (1) (1)

(ii)
- diagram showing double stranded DNA (1) and the position of primers (1) (2)



(iii)
- shorter primers have less combinations of base sequences (1)
- there is a high chance of annealing to wrong positions of the DNA strand (1)
- therefore, DNA strands of different sizes are amplified (2)

(iv)
- the *Agrobacterium* contains plasmids for target genes to be inserted (1)
- the *Agrobacterium* can infect crop cells (1) and transfer the genes of the plasmid to the genome of the crop cells (2)

(v)
- with the presence of gene K and hence its toxic proteins, the root system of transgenic crops is less likely to be attacked / damaged by insects (1)
- therefore their root system can:
  - absorb more nutrients / water from soil
  - provide better anchorage / support

  } any one (1)
  than their non-transgenic counterpart (3)
- therefore the crops can grow to a bigger size / grow better / support more fruit formation / store more food (1)

Applications in biotechnology

PP P2 4a

4. (a) (i) Bone marrow stem cells are capable of differentiating into lymphocytes so that the patients can be protected against infections. 1

(ii) Somatic gene therapy works by inserting a normal gene into the patient's cells to correct the genetic disorder by compensating for the malfunctioning gene (i.e. production of lymphocytes can be resumed in this case). 1
Advantage: no need to wait for a matching donor for the bone marrow cells / no problem of rejection 1

(iii) All the cells in the offspring will contain the normal gene if germ-line therapy is employed. However, only the body cells having the inserted gene and their daughter cells will have the normal gene in somatic gene therapy. 1
With germ-line gene therapy, the normal gene can be inherited by the subsequent generations. However, the normal gene obtained by somatic gene therapy is not inheritable. 1

(iv) The desired gene carried by the vector virus is randomly inserted into the patient's genome. 1
This desired gene may be inserted into the DNA sequence of another gene and affect the expression of that gene. 1

2012 P2 4b i, iii

(b) (i)
- the immune system may treat the transplanted organ as 'foreign', resulting in rejection (1) (1)
- have to wait for long time for a suitable donation / no enough donors (1) (1)

(ii)
- illegal to produce a human clone / wastage of many embryo during cloning / killing one person to help another person is morally not accepted (1) (1)

(iii) (1)
- bone marrow (1) / dermis of skin / umbilical cord blood or embryonic cells which were obtained long time ago (1)
other acceptable answers

(2)
- the stem cells proliferate to increase in cell number (1) (1)
- then they are differentiated into nerve stem cells (1) (1)
- which is introduced to Keith's body to repair the damaged tissue (1) (1)

4. (b) (iii) (3) Any *two* of the following: (max. 2)
- not all adult stem cells are identified (1)
- the conditions for culturing stem cells have not been figured out (1)
- some stem cell continue to proliferate after transplant and become cancer cells (1)
- the conditions needed to initiate the differentiation of stem cells into specialised cell types have not been figured out (1)

2013 P2 4a iii

(iii)
- due to the high growth rate of bacteria, the product yield from GM bacteria is much higher than that from animal pancreas because it takes long time to rear animals (1)
- insulin can be produced continuously from the bacterial culture whereas each animal can provide only a limited amount of animal pancreas (1) (max. 2)
- the cost of purification of insulin from bacterial culture is lower than that from the animal pancreas (1) as it is less complicated

2015 P2 4a (i→Immune system in compulsory part)

4. (a) (i) Any *two* of the following: (2)
- white blood cells are responsible for recognizing pathogens (1) / foreign cells
- and production of antibodies to fight against pathogens (1)
- and kill the pathogens (1)
- without these roles, the kid is more susceptible to infections (1)

(ii)
- cells from bone marrow are stem cells for producing white blood cells (1)
- once the working gene is inserted into these stem cells, the cells can divide and produce white blood cells that carry the working gene (1)
- hence, this provides a long-term cure to the disease (1#)
- on the other hand, if the working gene is inserted into mature white blood cells, the working gene is lost after their death (1), therapy has to be repeated from time to time (4)
- thus its effect is short-lived (1#)

(# mark to be awarded once)

(iii)
- a vector is used to carry the working ADA gene (1)
- it delivers and inserts a working ADA gene / normal gene into Nina's genome / cell (1) (3)
- this inserted gene is expressed to produce a functional protein / enzyme (1), which should overcome the defect

(iv) Any *two* of the following:
- genes may be inserted at random locations in the genome which may cause harmful mutations to the DNA (1) / failure of the expression of essential genes
- the target genes may insert regulatory sequences that trigger the expression of nearby genes leading to cancer (like leukaemia) (1) (2)
- some patients may show an immune reaction to the vector (1) and reject the gene product
- viral vector may regain the ability to cause diseases (1)

2016 P2 4a (i,ii→Nervous system in compulsory part)

4. (a) (i)
- the neurones in that particular area are responsible for producing neurotransmitters (1)
- and this type of neurotransmitters is vital for coordinating voluntary responses / muscle coordination / muscle contraction (1) (2)

(ii)
- since the drug has similar molecular structure as that of the neurotransmitter, they can stimulate the next neurone (1) / bind to the receptor site at the next neurones
- and elicit a nerve impulse (1) to mimic the effect of the neurotransmitter (2)

(iii)
- the transplanted stem cells differentiated into neurones in the patients' brain (1)
- and resume the function of producing the neurotransmitter when needed (1) (2)

(iv)
- the stem cells are derived from the patients' own tissue, there is no rejection / it will not trigger immune response after transplant (1)
- skin cells are actively dividing cells which are of unlimited supply (1)
- using aborted foetal tissue has political and ethical issues while deriving cells from adult skin cells do not (1) (3)

2018 P2 4a (i→circulatory & immune system)

4. (a) (i) Any *two* of the following: (2)
- it has to be performed regularly (1)
- risk of contracting infectious diseases via blood transfusion (1)
- iron accumulation / deposition in the liver may occur (1)

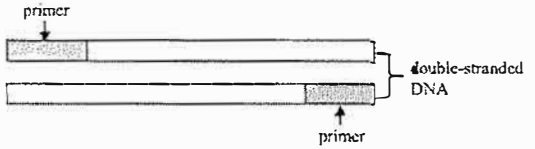
(ii) (1)
- stem cells have the ability to divide and reproduce new cells (1)
- differentiate into liver cells with normal functions / so cells with ability to produce the blood clotting protein will be produced in John for his lifetime (1) (2)

(2)
- as the transformation / transfer of the gene by viral vector was carried out outside John's body (1)
- the risk of having the viral vector triggering immune response in John is removed (1)
- cell culture allows the selection of transfected cells which can successfully produce the protein for transplantation / which are free from other abnormalities (1)
- this eliminates the risk of having the gene inserted into the wrong position and fails to express / causing cancers / disrupting other functioning of the cells (1) (4)

2019 P2 b iv,v

(b) (i)
- primer annealing (1) (1)

(ii)
- diagram showing double stranded DNA (1) and the position of primers (1) (2)



(iii)
- shorter primers have less combinations of base sequences (1)
- there is a high chance of annealing to wrong positions of the DNA strand (1)
- therefore, DNA strands of different sizes are amplified (2)

(iv)
- the *Agrobacterium* contains plasmids for target genes to be inserted (1)
- the *Agrobacterium* can infect crop cells (1) and transfer the genes of the plasmid to the genome of the crop cells (2)

(v)
- with the presence of gene K and hence its toxic proteins, the root system of transgenic crops is less likely to be attacked / damaged by insects (1)
- therefore their root system can;
  - absorb more nutrients / water from soil
  - provide better anchorage / support

  } any one (1)
  than their non-transgenic counterpart
- therefore the crops can grow to a bigger size / grow better / support more fruit formation / store more food (1) (3)

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(ii)
- illegal to produce a human clone / wastage of many embryo during cloning / killing one person to help another person is morally not accepted (1) (1)