AS 9 **GENETIC ENGINEERING**

QUESTIONSHEET 1

Do not write in margin

Read through the following account of genetic engineering and then fill in the spaces with the most appropriate word or words.

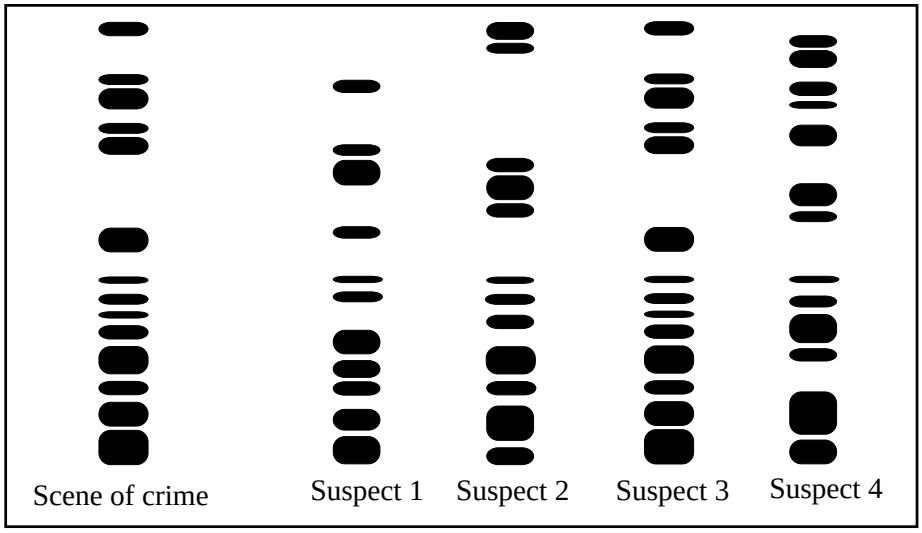
During the process of hormone manufacture by genetic engineering, human RNA is extracted and converted to single stranded DNA by treatment with ..... . This is then treated with ..... to produce double stranded (double helix) DNA. Plasmid DNA is also extracted from suitable bacteria for use as a ..... .The human and plasmid DNAs are then treated separately with ..... which cuts them into fragments which have the same complementary ..... .The fragments of human and plasmid DNA are then mixed with ..... . This joins the two types of DNA together as ..... DNA which will hopefully contain the gene required for hormone synthesis. The plasmids are then mixed with host bacterial cells, such as cells of ..... . The presence of the chemical ..... aids the plasmid uptake by the bacteria. The bacteria can then undergo large scale culture and should produce suitable quantities of the required hormones. Hormones made in this way are.....and ..... .

TOTAL / 11 _____

Do not write in margin

QUESTIONSHEET 2

The diagram shows the results of a DNA fingerprint analysis using a microscopic blood sample found at the scene of a crime. The DNA profile of the blood is shown on the left and labelled 'scene of crime'. DNA profiles produced using blood samples from four suspects are shown on the right.



(a) (i) Which suspect has been incriminated by the DNA analysis?

..... [1]

(ii) Give a reason for your answer.

..... [1]

(b) Briefly describe the part played by each of the following in the production of the DNA profiles:

(i) polymerase chain reaction.

..... [1]

(ii) restriction endonucleases.

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..... [2]

(iii) gel electrophoresis.

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..... [2]

(iv) radioactive DNA probes.

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..... [3]

TOTAL / 10

QUESTIONSHEET 3

Recombinant DNA products can be made either from genetically modified cloned cells such as bacteria or from genetically modified mammalian cells in tissue culture.

(a) Name three recombinant DNA products that are manufactured for medical use.

1 .....

2 .....

3 .....

[3]

(b) Suggest three advantages of producing genetically modified products from cloned bacterial cells rather than from tissue cultures of mammalian cells.

1 .....

2 .....

3 .....

[3]

(c) Recombinant DNA products can also be produced in transgenic animals. For instance, alpha-1 antitrypsin can be produced by transgenic sheep. Suggest an advantage of producing a recombinant DNA product from a sheep rather than from a bacterial culture.

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.....

[2]

TOTAL / 8

GENETIC ENGINEERING

QUESTIONSHEET 4

Do not write in margin

A genetic disorder in humans is alpha-1 antitrypsin deficiency. The gene coding for alpha-1 antitrypsin mutates with the result that the liver fails to manufacture this enzyme. The function of alpha 1-trypsin is to destroy any unwanted protease enzymes in body tissues. If the enzyme is missing then the main symptom is degeneration of alveolar tissue in the lungs due to attack by proteases. This leads to the condition of 'inherited emphysema'.

The disorder can be treated either by gene therapy or by dosage with alpha-1 antitrypsin. It is possible to use transgenic sheep to provide adequate quantities of alpha-1 antitrypsin to enable patients to be treated.

DNA from sheep mammary gland cells, human DNA containing the alpha-1 antitrypsin gene and DNA containing a gene for neomycin resistance are combined as recombinant DNA. This DNA construct is placed into sheep cells which are then cultured in large numbers. The cells are then treated with neomycin and the surviving cells are used as nuclear donors to transfer the human alpha-1 antitrypsin gene into transgenic sheep.

(a) (i) How is human DNA usually obtained for use in gene technology?

.....

.....

[2]

(ii) How is recombinant DNA made from the sheep and human DNA?

.....

.....

.....

[3]

(iii) How is the DNA construct placed into sheep cells.

.....

.....

[2]

(iv) Why are the cells treated with neomycin?

.....

.....

[2]

(b) If the insertion of the recombinant DNA into the transgenic sheep is successful then the sheep should start to secrete large quantities of human alpha-1 antitrypsin from its liver.

(i) How may alpha-1 antitrypsin be obtained from the sheep?

.....

[1]

(ii) How could the alpha-1 antitrypsin be introduced to the lungs during treatment?

.....

[1]

TOTAL / 11 _____

Do not write in margin

QUESTIONSHEET 5

The two commonest hereditary lung diseases in individuals of European descent are alpha-1 antitrypsin deficiency and cystic fibrosis. Both of the human genes involved have been cloned and gene therapy is of potential use in the treatment of both diseases. **line 2**

Cystic fibrosis is due to a mutation in a gene that codes for a chloride channel protein in the cell membranes of epithelial cells. This protein regulates the secretion of chloride ions from the epithelial cells. If the secretion of chloride ions is reduced then the membrane resting potentials are raised, the surfaces are inadequately moistened with tissue fluid and mucus accumulates. This becomes infected and inflammation occurs. The symptoms affect the lungs, liver, gastrointestinal tract and pancreas. Since 90% of deaths due to cystic fibrosis are because of respiratory failure, gene therapy has focussed on correcting the genetic defect in the lungs.

(a) (i) What is meant by the phrase 'the human genes have been cloned' (line 2)?

.....

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[2]

(ii) List the sequence of steps involved in cloning, naming any enzymes that are used.

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[5]

(b) In gene therapy the cloned genes must be delivered to the body organs that need them. In the case of cystic fibrosis this is the lungs. The genes must be inserted into suitable vectors for delivery.

(i) Suggest two suitable vectors that are used to transport cloned genes to lung cells.

1 ..... 2 .....

[2]

(ii) Suggest two ways to introduce the vectors to the lungs.

1 ..... [1]

2 ..... [1]

(c) Alpha-1 antitrypsin is an enzyme that destroys protease enzymes in body tissues. It is manufactured by the liver cells but its most important site of operation is in the lungs. If alpha-1 antitrypsin is deficient then lung tissue is damaged by protease activity and hereditary emphysema develops. In gene therapy of this disease which would be the target organ for the gene vectors? Give a reason for your answer.

Organ ..... [1]

Reason ..... [1]

TOTAL / 13

QUESTIONSHEET 6

Do not write in margin

Is genetical modification of crop plants for insect and pesticide resistance the answer to the world's food problems or could it lead to a potential ecological disaster?

The European Corn Borer insect costs American farmers more than $1,000,000 in losses per year.

The first gene transplant into a plant by recombinant DNA technology was made over 15 years ago. Recombinant DNA technology enables geneticists to introduce new variations into a wide range of organisms.

Many transgenic plants planted in American corn and cotton fields were supposed to give the plants 'do-it-yourself' resistance to insect attack. The implanted gene was taken from a soil bacterium, Bacillus thuringiensis, which is a pathogen to many species of insects. The gene manufactures a protein (referred to as Bt) which damages the gut of chewing insects, resulting in their death. The transgenic corn and cotton plants produce Bt in adequate quantities to kill insects that eat the crop. Mammals, fish and beneficial insects that eat crop pests are unharmed.

For several years in America, farmers have bought and planted genetically modified Bt corn and cotton seed, and as a result have obtained greatly increased yields, and saved large amounts of money by not using insecticides. However, in 1996 more than 18,000 acres of Bt crops were overwhelmed and devastated by insects.

In 1998 Bt modified corn was found to be releasing pollen which contained the Bt protein. When this pollen landed on milkweed plants, the food plant of the Monarch Butterfly caterpillar, it was found that caterpillers which had eaten the contaminated milkweed died within four days.

(a) Outline the steps which would be used to transfer the Bt gene from Bacillus thuringiensis into cotton plants.

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[4]

(b) Outline the steps by which the Bt gene enables the cotton plant to synthesize the Bt protein.

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[4]

(*Continued...*)

Do not write in margin

(c) Some insects have already developed resistance to the Bt protein since Bt crop damage due to insect attack is occuring. Suggest a mechanism by which Bt resistance may have developed in insects.

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.....

[4]

(d) Suggest two advantages and two possible disadvantages of introducing insecticide resistance into crop plants.

Advantages:

1 .....

2 .....

[2]

Disadvantages:

1 .....

2 .....

[2]

(e) Comment on the ethical issues involved in the introduction of new varieties of crop plants by gene technology.

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.....

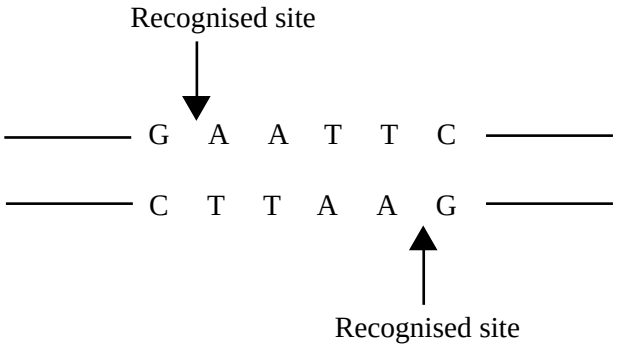
[2]

TOTAL / 18

QUESTIONSHEET 7

Do not write in margin

Restriction enzymes are so-called because they cut DNA at specific 'restricted' sites known as recognition sites. The recognition site and the cleavage products of the restriction enzyme EcoRL are shown below.



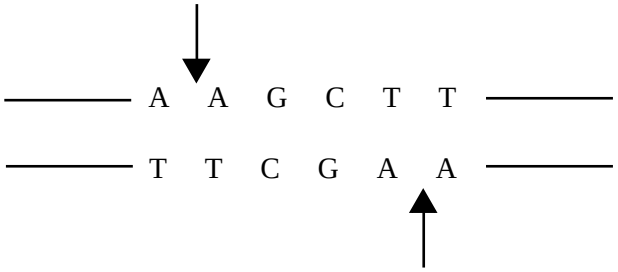
cleavage products

G A A T T C

C T T A A G

(a) (i) The recognition site of the restriction enzyme Hind III is shown below. Write down the cleavage products in the space provided.

cleavage products



[2]

(ii) Using the example given in (a) (i) explain what is meant by the term 'sticky ends'

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[3]

(iii) Outline the significance of sticky ends in genetic engineering.

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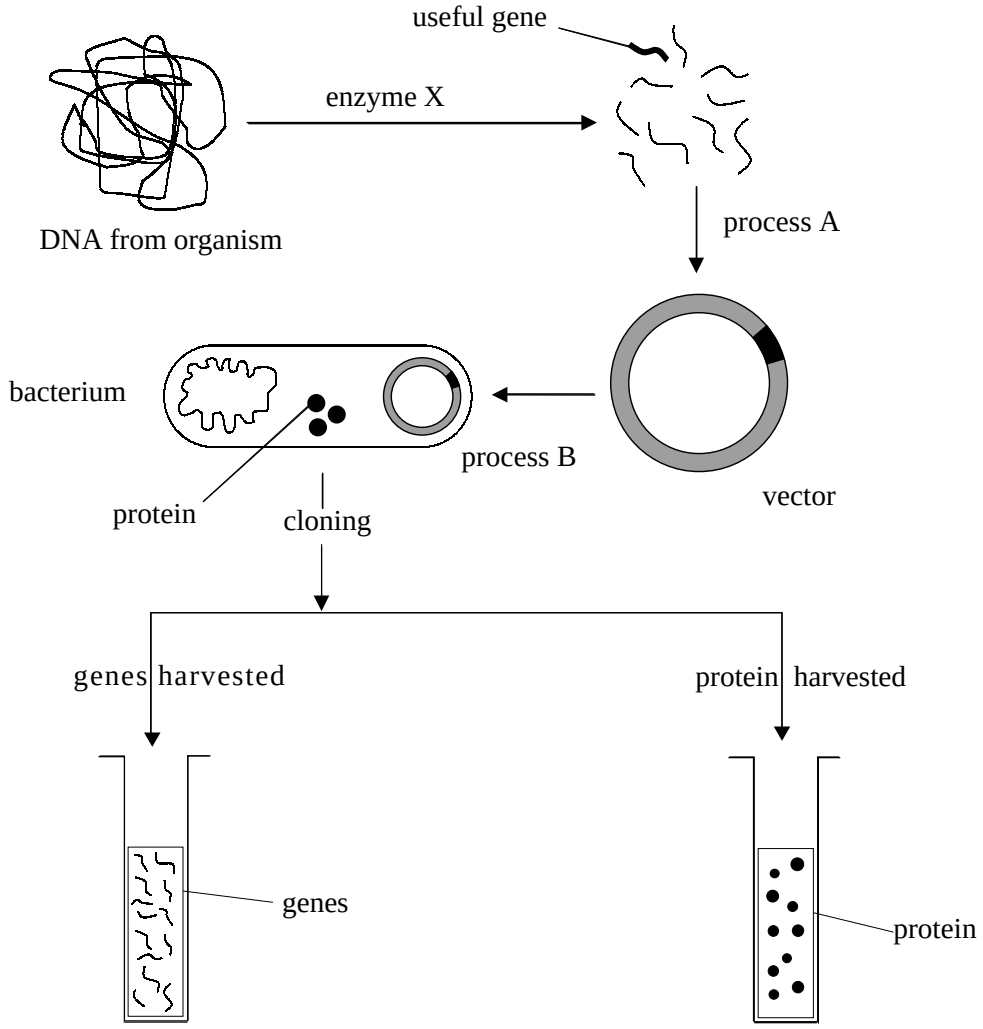
[2]

TOTAL / 7

QUESTIONSHEET 8

Do not write in margin

The diagram below shows an outline of the processes involved in the production of useful genes and proteins by genetic engineering.



(a) (i) Name enzyme X

..... [1]

(ii) Name the enzyme which will be used to open the vector

..... [1]

(b) What is the likely nature of the vector?

..... [1]

(c) Outline process B

.....

.....

..... [3]

(d) Outline the process which takes place inside the bacterium

.....

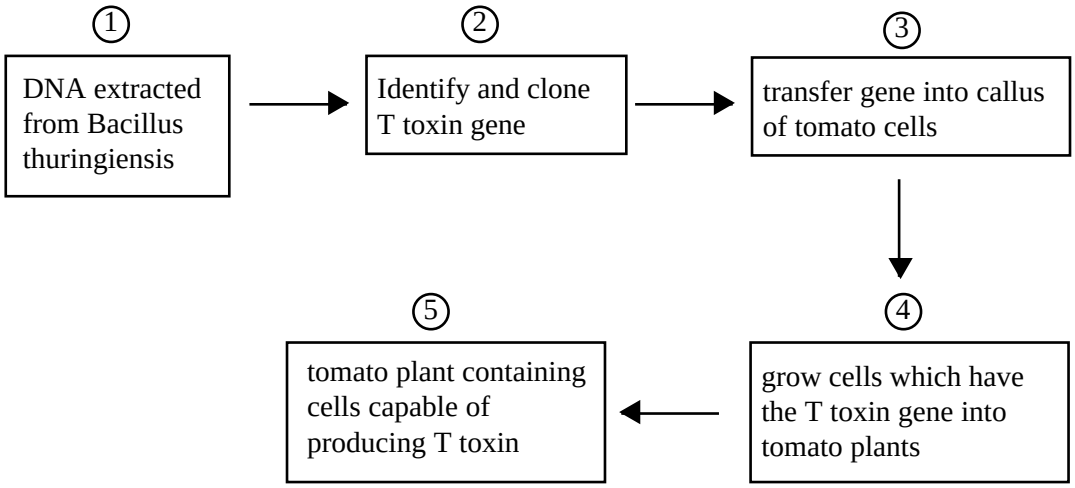
..... [2]

TOTAL / 8

QUESTIONSHEET 9

Do not write in margin

Bacillus thuringiensis (Bt) is a bacterium that lives naturally in the soil. Bt produces a toxic protein known as T toxin which is poisonous to caterpillars and some other insects. Genetic engineers have inserted the gene for the production of T toxin into tomato plants. An outline of the procedure which they used is given below.



(a) What is the purpose of inserting the T toxin into tomato plants?

..... [1]

(b) Explain how scientists could have identified the T toxin gene.

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..... [3]

(c) Name one enzyme which would have been used during cloning at stage 2.

..... [1]

(d) The scientists were not sure that all of the leaves of the tomato plants would produce the toxin. Outline how this could be investigated in a laboratory.

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..... [3]

TOTAL / 8

QUESTIONSHEET 10

Do not write in margin

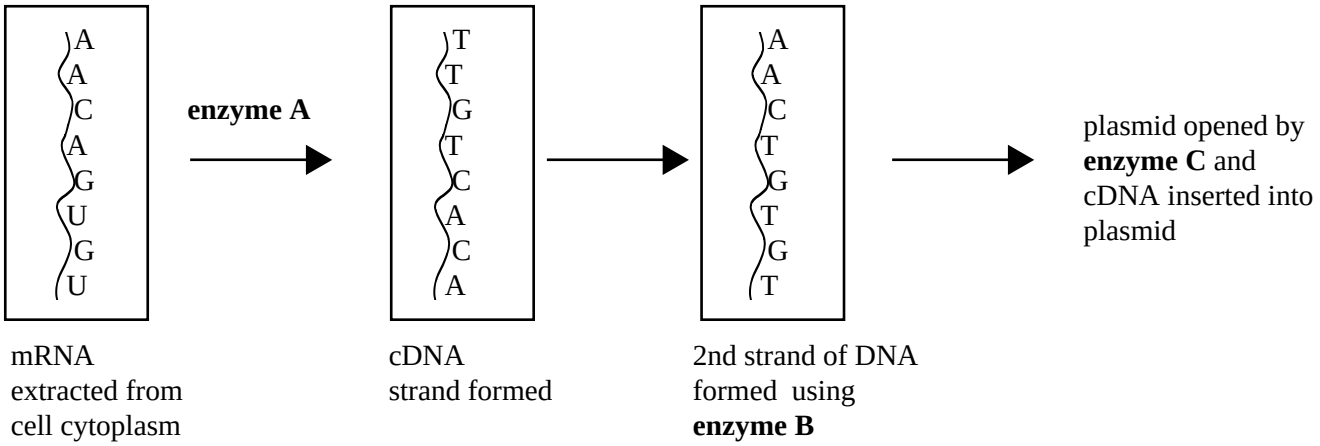
In an effort to mass produce a useful protein, scientists extracted a precise section of mRNA from the cytoplasm of cells which naturally produced the protein.

The mRNA strand was then used as a template to enzymatically synthesise a strand of complementary DNA (cDNA).

A 2nd enzyme was then used synthesise a strand of DNA complementary to the first DNA strand.

The cDNA fragment was then inserted into a plasmid. To do this the plasmid was opened using enzymes and the sticky ends of the plasmid were joined to the complimentary bases on the cDNA.

3.



(a) Identify the enzymes A B and C.

A. .....

B. .....

C. ..... [3]

(b) Identify the vector which was used in this investigation.

..... [1]

(c) Name one other type of vector.

..... [1]

(d) The vector was then inserted into the bacterium E.coli for mass production of the protein.

(i) How could the vector be inserted into the bacterium?

.....

..... [2]

(ii) How could the protein be mass produced?

.....

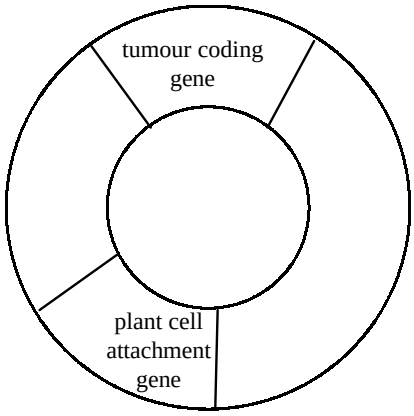
..... [2]

TOTAL / 9

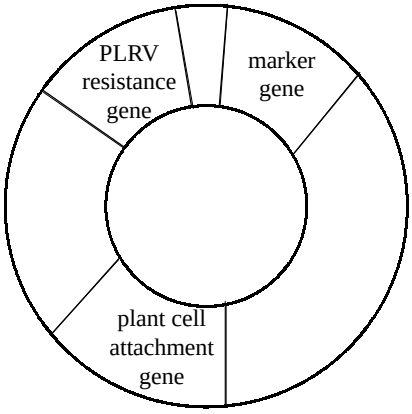
GENETIC ENGINEERING

QUESTIONSHEET 11

Genetic engineering is used to 'improve' many crop plants. One example is the introduction of a gene into potato plants which gives them resistance to infection by potato leafroll virus (PLRV). The tumour inducing (Ti) plasmid of Agrobacterium tumefaciens is used as a vector. The diagrams below show the Ti plasmid in its natural state and in its recombinant state.



natural state



recombinant state

(a) (i) How could large quantities of DNA fragments which contain the required gene be obtained?

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..... [6]

(ii) How could Ti plasmid fragments with the tumour causing genes cut out be obtained?

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..... [4]

(*Continued...*)

Do not write in margin

(iii) How could recombinant Ti plasmids be obtained?

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..... [2]

(iv) How could the plasmids be introduced into Agrobacterium tumefaciens?

.....

..... [2]

(v) How could the spliced genes be inserted into potato plants?

.....

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..... [3]

(b) The potato plants modified above are known as transgenic plants. State <u>two</u> other applications of transgenic plants.

1. .....

2. ..... [2]

GENETIC ENGINEERING

QUESTIONSHEET 12

Do not write in margin

Briefly describe the following:

(a) a gene probe:

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..... [4]

(b) the polymerase chain reaction:

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..... [6]

(c) DNA fingerprinting:

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..... [5]

TOTAL / 15

GENETIC ENGINEERING

QUESTIONSHEET 13

The table below refers to the uses of several enzymes which are asociated with genetic engineering. Complete the table by writing the names of an appropriate enzyme in each empty box.

Action of enzyme	Named enzyme
Enables transcription of DNA from mRNA	
Enables transcription of mRNA from DNA	
Cuts DNA at specific base sequences	
Binds DNA fragments of different origin together	
Enables polypeptide synthesis from amino acids in the ribosomes	
Enables DNA replication in the cell cycle	
Used to make multiple copies of DNA in genetic engineering	

TOTAL / 7

QUESTIONSHEET 14

Do not write in margin

Biologists have traditionally recognised three different subspecies of chimpanzee based on physical characteristics, genetics and geography. *Pan troglodytes troglodytes* lives in Central Africa, *Pan troglodytes schweinfurthei* lives in East Africa and *Pan troglodytes verus* lives in West Africa. Recently, a small new population of chimpanzees living in southeastern Nigeria has come to the attention of biologists. It is suspected that this small population make up a new subspecies. Genetic analysis of animals for taxonomic purposes is usually carried out on mitochondrial DNA.

(a) (i) Name a technique which could be used to compare the mitochondrial DNA of the different chimpanzee subspecies.

..... [1]

(ii) Briefly describe the technique you have named.

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.....

.....

..... [5]

(iii) What results would you expect to see if the Nigerian chimpanzees were a distinct subspecies?

.....

..... [2]

(b) Name two other applications of the technique you have described.

1. .....

2. ..... [2]

TOTAL / 10