

## Stage 2 Biology

# DNA and Proteins

## SASTA Revision Guide Questions (a selection)

### How to use:

- 1 – Sit down in 10-15min sessions (regularly) and complete a range of questions from the various subtopics (don't start at the beginning and work forward).
- 2 – Go to the end to find the answer – look for the question code at the back (e.g. E15)
- 3 – For any concepts that you got incorrect, go back and revise.

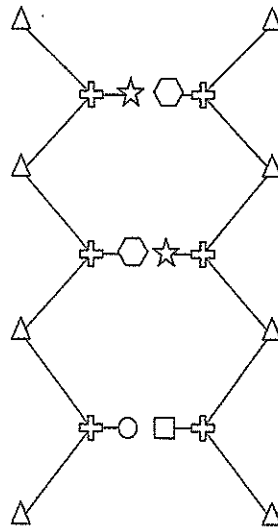


- A3 Which one of the following alternatives correctly identifies the type of sugar, the structure, and the bases found in a DNA molecule?

	Type of sugar	Structure	Bases
J.	Deoxyribose	Double stranded	A, T, C, G
K.	Deoxyribose	Single stranded	A, U, C, G
L.	Ribose	Double stranded	A, T, C, G
M.	Ribose	Single stranded	A, U, C, G

[2007 Q1]

- A4 Refer to the following diagram, which shows a segment of DNA:



Which of the following combinations identifies the symbol shown in the diagram above?

	+	△	⬡	☆	○	□
J.	sugar	phosphate	thymine (T)	adenine (A)	cytosine (C)	guanine (G)
K.	phosphate	sugar	guanine (G)	cytosine (C)	adenine (A)	thymine (T)
L.	sugar	phosphate	cytosine (C)	thymine (T)	guanine (G)	adenine (A)
M.	phosphate	sugar	adenine (A)	guanine (G)	thymine (T)	cytosine (C)

[2013 Q4]

- A5 Which one of the following cellular processes involves the pairing of the bases cytosine, thymine, and adenine with the bases guanine, adenine, and thymine respectively?

- J. Translation.
- K. Transcription.
- L. The replication of DNA.
- M. The bonding of amino acids.

[Adapted, 2001 Q1]

- A6 Which one of the following alternatives correctly identifies the packaging, shape and location of a chromosome in a prokaryotic cell?

	Packaging	Shape	Location
J.	Bound to proteins	Linear	Nucleus
K.	Bound to cytosol	Circular	Mitochondria
L.	Unbound	Linear	Nucleus
M.	Unbound	Circular	Cytosol

- A7 Refer to the following diagram, which shows a non-radioactive DNA strand and a radioactive DNA strand:



non-radioactive DNA strand



radioactive DNA strand

A cell with non-radioactive DNA underwent cell division in a medium containing radioactive nucleotides.

Which one of the following diagrams, J, K, L, or M, best represents the chemical composition of DNA molecules in the two resulting daughter cells?



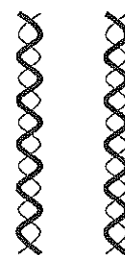
J.



K.



L.



M.

[2004 Q6]

- A8 Which one of the combinations in the table in Source 1 correctly identifies a cell type, the structure of its DNA, and the location of its DNA in the cell?

	Cell type	Structure of DNA	Location of DNA in the cell
J.	prokaryotic	linear chromosomes without proteins	cytosol
K.	prokaryotic	circular chromosomes with proteins	cytosol
L.	eukaryotic	linear chromosomes with proteins	nucleus
M.	eukaryotic	circular chromosomes without proteins	nucleus

[2020 Q1]

## 4.2 GENES AND PROTEIN SYNTHESIS

- B1 Erythromycin is an antibiotic that interferes with the process of protein synthesis in bacterial cells.

It is possible that erythromycin would affect the

- J. transcription of mRNA in the nucleus of the bacterial cell.
- K. attachment of amino acids to mRNA.
- L. movement of ribosomes along mRNA.
- M. attachment of ribosomes to the endoplasmic reticulum of the bacterial cell.

[2010 Q3]

- B2 On a strand of mRNA, 30% of the bases are uracil (U), 10% of the bases are cytosine (C), and 20% of the bases are adenine (A). The mRNA strand was transcribed from a strand of DNA. What percentage of the bases on this strand of DNA would be cytosine?

- J. 10%
- K. 20%
- L. 30%
- M. 40%

[2005 Q6]

- B3 Refer to the following sequence of nucleotide bases which represents a section of an mRNA molecule:

UAC CCG AAU UAG

Which one of the following sequences of nucleotide bases would be found on the strand of DNA that was transcribed to produce this section of an mRNA molecule?

- J. TAC CCG AAT TAG.
- K. UAC CCG AAU UAG.
- L. AUG GGC UUA AUC.
- M. ATG GGC TTA ATC.

[2014 Q2]

- B4 A length of mRNA attached to a ribosome codes for the production of a polypeptide that is 120 amino acids long.

How many mRNA nucleotides code for the amino acids in this polypeptide?

- J. 40
- K. 120
- L. 360
- M. 720

[2007 Q4]

- B5 Small pieces of RNA, called RNA interference (RNAi), can prevent the expression of specific genes. To do this they must first enter a cell.

RNA molecules are negatively charged and therefore cannot readily dissolve in lipids.

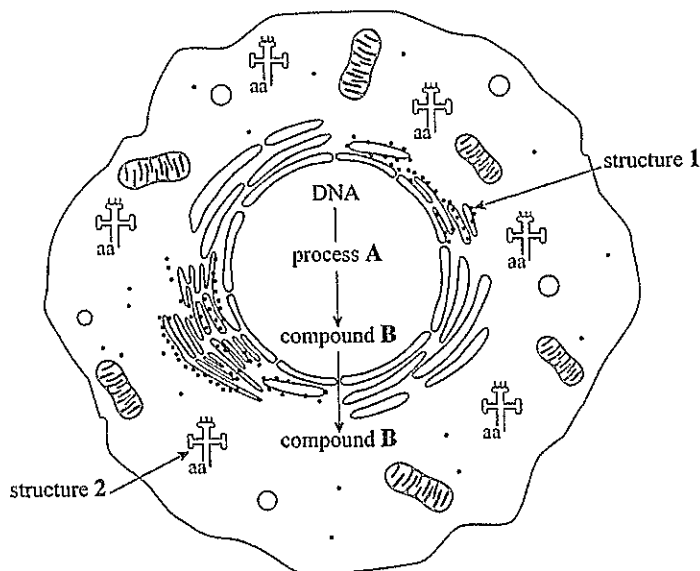
RNAi molecules can be modified to be smaller and to have a reduced negative charge, thus making them lipid-soluble.

Modified RNAi, but *not* other RNA

- J. could enter a cell through the membrane.
- K. could attach to ribosomes.
- L. would be produced by transcription.
- M. may contain thymine.

[2010 Q6]

B10 Refer to the following diagram, which shows process A, compound B, and structures 1 and 2 in a cell.



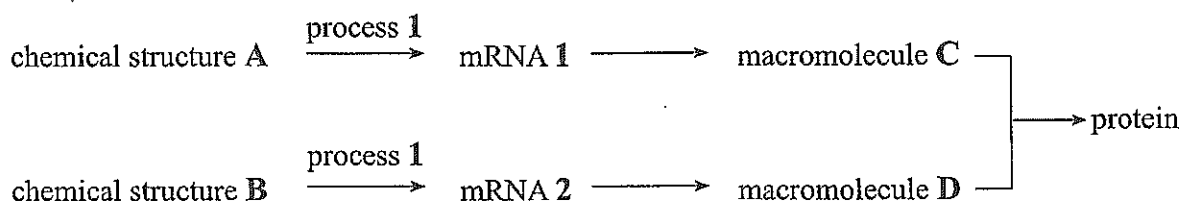
[This diagram is not drawn to scale.]

Which one of the following combinations identifies process A, compound B, and structures 1 and 2?

	Process A	Compound B	Structure 1	Structure 2
J.	Transcription	mRNA	Ribosome	tRNA
K.	Transcription	mRNA	Amino acid	Anticodon
L.	DNA replication	DNA	Ribosome	Anticodon
M.	DNA replication	DNA	Amino acid	tRNA

[2009 Q5]

B11 Refer to the following diagram, which shows some of the steps involved in the manufacture of a protein in a eukaryotic cell:



Which one of the following combinations correctly matches the name of chemical structure A, the location of process 1, and the name of macromolecule D?

	Name of chemical structure A	Location of process 1	Name of macromolecule D
J.	gene	nucleus	amino acid
K.	amino acid	cytoplasm	polypeptide
L.	polypeptide	cytoplasm	amino acid
M.	gene	nucleus	polypeptide

[2006 Q4]

B15 Refer to the following table, which shows mRNA codons for amino acids:

First base in sequence ↓	Second base in sequence				Third base in sequence ↓
	U	C	A	G	
U	phenylalanine phenylalanine leucine leucine	serine serine serine serine	tyrosine tyrosine — —	cysteine cysteine tryptophan —	U C A G
C	leucine leucine leucine leucine	proline proline proline proline	histidine histidine glutamine glutamine	arginine arginine arginine arginine	U C A G
A	isoleucine isoleucine isoleucine methionine	threonine threonine threonine threonine	asparagine asparagine lysine lysine	serine serine arginine arginine	U C A G
G	valine valine valine valine	alanine alanine alanine alanine	aspartic acid aspartic acid glutamic acid glutamic acid	glycine glycine glycine glycine	U C A G

The base sequence U A U on

- J. tRNA codes for tyrosine.
- K. mRNA codes for isoleucine.
- L. DNA codes for tyrosine.
- M. tRNA codes for isoleucine.

[2012 Q4]

B16 The product of *gene 1* is a protein that binds to a promoter region on the DNA near *gene 2*. This results in the expression of *gene 2*.  
A mutation in which one of the following will most likely prevent the expression of *gene 2*?

- J. An intron of *gene 1*.
- K. An exon of *gene 1*.
- L. The product of *gene 1*.
- M. The product of *gene 2*.

[2018 Q3]

B17 The base sequence AGC on the coding strand of a DNA molecule codes for an amino acid. Identify its corresponding anticodon.

- J. UCG
- K. AGC
- L. TCG
- M. UGC

[2020 Q2]

B18 A gene codes for

- J. complementary base pairs on DNA molecules.
- K. an RNA molecule.
- L. sequences of polypeptides on DNA molecules.
- M. an amino acid molecule.

[2012 Q3]

B22 Micro RNA (miRNA) molecules are RNA molecules that are about 21 to 23 nucleotides long. These miRNA molecules are transcribed from DNA but are not translated. In bacteria they are known to be complementary to part of one or more messenger RNA (mRNA) molecules to which they can bind.

- (a) State *one* structural difference between DNA and mRNA.  
 (b) State the complementary mRNA nucleotide sequence for the following miRNA nucleotide sequence by writing the appropriate letters in the boxes below.

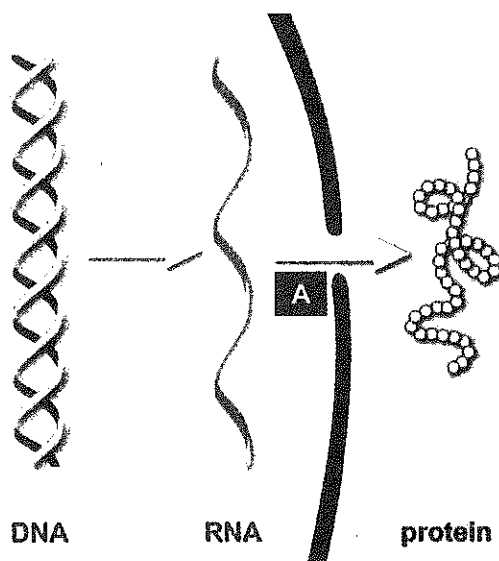
miRNA nucleotide sequence	A	G	G	C
complementary mRNA nucleotide sequence	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

- (c) State the role of miRNA in protein synthesis.  
 (d) Explain how the binding of an miRNA molecule to an mRNA molecule will affect the role of the mRNA.

[Adapted, 2008 Q32]

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B23 Refer to the following diagram, which shows cellular process A:

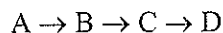


- (a) State the name of cellular process A (RNA → protein).  
 (b) Explain what the DNA coding strand codes for.  
 (c) Describe the roles of mRNA and tRNA in the process of protein synthesis in eukaryotic cells.  
 mRNA:  
 tRNA:  
 (d) The diagram above does not illustrate the formation of a mature strand of mRNA.  
 (i) State one difference between the mature and immature mRNA strand.  
 (ii) Describe how a mature strand of mRNA is formed.  
 (iii) State why only parts of the immature RNA strand are translated to form a polypeptide.

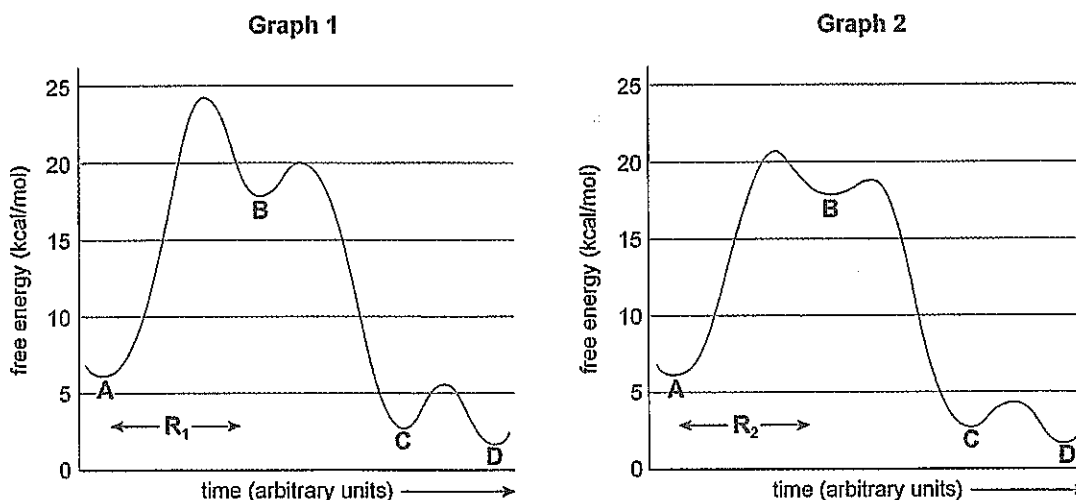
[Adapted, 2011 Q26]

### 4.3 PROTEIN STRUCTURE AND FUNCTION

C1 The following metabolic pathway occurs in some cells:



Refer to the following graphs, which show the changes in free energy along this pathway. One graph shows the changes in free energy in the presence of enzymes and the other shows the changes in free energy in the absence of enzymes.



Which one of the following statements is *not* consistent with the data presented for the reactions in the graphs above?

- J. Reaction  $R_2$  is an enzyme-catalysed reaction in which product B was synthesised from substrate A.
- K. Product C contains less free energy than either substrate A or substrate B.
- L. In the absence of enzymes the formation of product B requires less activation energy than is required to break down product C.
- M. The formation of product C requires the prior synthesis of substrate B.

[2015 Q4]

C2 Angiotensin II is a protein produced by healthy human beings. It binds to the receptor in the blood vessels and causes the blood vessels to constrict, resulting in a rise in blood pressure. Irbesartan is a drug that is used to reduce high blood pressure by blocking the angiotensin II receptors in the walls of the blood vessels.

The information above suggests that irbesartan molecules

- J. have a very similar shape to the angiotensin II receptors.
- K. cause the angiotensin II receptor to denature.
- L. have a shape very similar to that of the angiotensin II molecules.
- M. have a complementary shape to the angiotensin II molecules.

[2013 Q3]

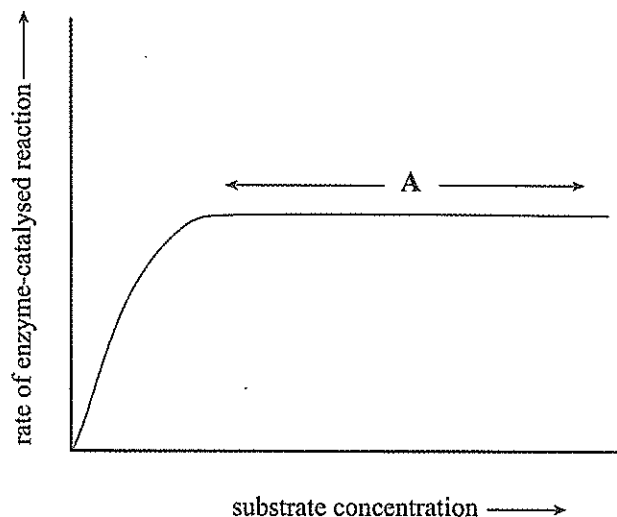
C3 The shape of the active site of an enzyme molecule can be altered by

- J. lowering the activation energy.
- K. the presence of molecules with a shape similar to that of the active site.
- L. the presence of molecules with a shape complementary to that of its substrate.
- M. increasing the temperature.

[2009 Q3]



- C11 Refer to the following graph, which shows the results of an experiment to determine the effect of varying the substrate concentration on the rate of an enzyme-catalysed reaction. The experiment was conducted at the optimum pH for the enzyme and in the presence of an inhibitor:

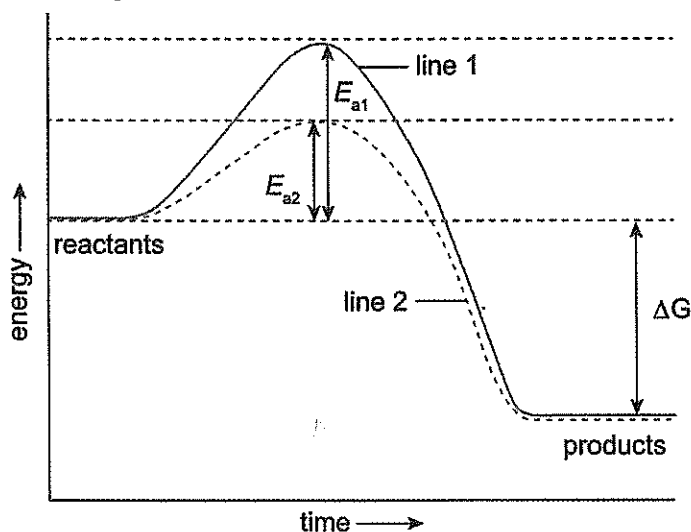


The rate of the enzyme-catalysed reaction in the section labelled A on the graph could be higher if

- J. the substrate concentration was increased.
- K. the pH was increased.
- L. the enzyme concentration was decreased.
- M. the inhibitor concentration was decreased.

[2006 Q3]

- C12 Refer to the following graph, which shows the energy changes during a chemical reaction in a cell. Line 1 and line 2 represent the same reaction under different conditions.

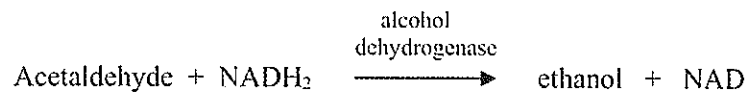


Which one of the following statements is consistent with the information in the graph above?

- J. The difference between  $E_{a1}$  and  $E_{a2}$  is called the 'activation energy'.
- K. Line 1 shows the energy changes during the chemical reaction in the absence of an enzyme.
- L.  $\Delta G$  indicates that the reaction requires energy.
- M. In this reaction the energy required to break bonds is greater than the energy released when new bonds are formed.

[2011 Q6]

- C21 Refer to the following equation which shows the final steps in a metabolic pathway. Assume that there is always some alcohol dehydrogenase available to catalyse the reaction.

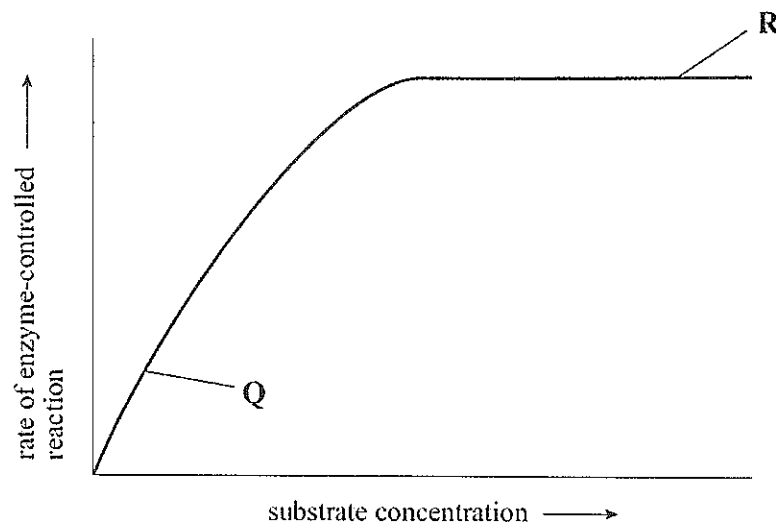


Which one of the following statements is **incorrect**?

- J. The rate of production of ethanol and NAD may depend on the concentration of alcohol dehydrogenase
- K. The rate of production of ethanol and NAD may depend on the temperature of the solution
- L. The final concentration of ethanol and NAD produced may depend on the initial concentration of acetaldehyde.
- M. The final concentration of ethanol and NAD produced may depend on the concentration of alcohol dehydrogenase.

[2001 Q7]

- C22 Refer to the following graph, which shows the effect of increasing substrate concentration on the rate of an enzyme-controlled reaction. All other quantities in the experiment were kept constant.



It is reasonable to conclude that at

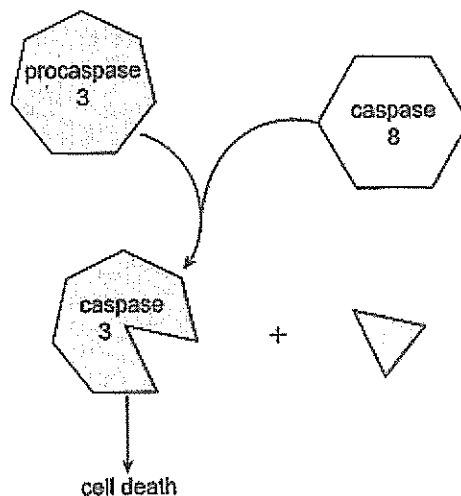
- J. Q the increase in the rate of reaction is due to an increase in enzyme concentration.
- K. Q there are unoccupied active sites on the substrate.
- L. R the rate of reaction may be limited by enzyme concentration.
- M. R the reaction has stopped because the substrate concentration is too high. [2003 Q4]

- C23 Which one of the following statements is correct?

- J. The primary structure of proteins is affected by any change in temperature.
- K. The secondary structure of proteins is made up of more than one polypeptide.
- L. The tertiary structure of proteins determines the shape of the active site of an enzyme.
- M. The quaternary structure of amino acids is affected by a change in pH.

[2019 Q2]

- C27 Caspase 3 is an enzyme that is responsible for the final stages of controlled cell death. It is produced in an inactive form called 'procaspase 3', which is activated by caspase 8, as illustrated in the diagram below.



- (a) State the name of the substrate for caspase 8  
 (b) State *one* reason why procaspase 3 is produced in an inactive form in cells.  
 (c) Explain how caspase 8 increases the rate of activation of procaspase 3.  
 (d) There are many inhibitors of caspases.  
 Explain *one* way in which an inhibitor of caspase 8 could reduce the rate of activation of procaspase 3.

[2018 Q17]

- C28 When soft-centred chocolates are made, the enzyme invertase is used to convert sucrose into the smaller molecules glucose and fructose. This causes the centres of the chocolates to become softer and sweeter.

- (a) State why the invertase must not be heated to temperatures above 45 °C.  
 (b) State how invertase increases the rate of conversion of sucrose into glucose and fructose.  
 (c) Explain why the effect of an inhibitor on an enzyme may be reduced by increasing the concentration of the substrate.

[2004 Q26]

- C29 Colorectal cancer is a common disease worldwide. Studies have shown that changes to DNA methylation patterns in a range of tumour suppressor genes (TSGs) can trigger the development of colorectal cancer.

DNA methyltransferases (DNMTs) are enzymes that catalyse the addition of methyl groups to DNA. DNMTs are required to maintain methylation patterns from parent cells to daughter cells in the process of cell division.

Researchers are developing epigenetic therapies that target DNMTs. A group of drugs, called DNA methyltransferase **inhibitors** (iDNMTs), have been developed to treat colorectal cancer.

- (a) Explain **one** way in which iDNMTs may affect the function of DNA methyltransferases (DNMTs).  
 (b) Explain why iDNMTs may cause side effects.

[2022 Q16 (d) and (e)]

## 4.4 PHENOTYPIC EXPRESSION AND MUTATIONS

D1 Refer to the following table, which shows some of the mRNA codons for some amino acids:

mRNA codon	Amino acid
AUG	methionine
AAG	lysine
GGC, GGU	glycine
UUU, UUA	phenylalanine
AUA	isoleucine
UAA, UAG	stop

A section of mRNA has the following codons:

codon number	.....	51	52	53	54	55	.....
mRNA codon	.....	GGC	AAG	UUU	AUA	AAG	.....

Which one of the following changes in the mRNA section shown above would *not* result in the production of a different protein?

- J. The deletion of the third U in codon 53.
- K. The substitution of U for the second A in codon 52.
- L. The insertion of U between codons 51 and 52.
- M. The substitution of U for C in codon 51.

[2006 Q5]

D2 Which one of the following statements is not supported by current evidence?

- J. A mutation may have no effect on future generations.
- K. A mutation can be brought about by environmental factors.
- L. A mutation is usually of benefit to the organism that inherits it.
- M. A mutation may involve a change in gene structure.

[2004 Q2]

D3 In human DNA, which nucleotide base is most commonly methylated?

- J. Adenosine
- K. Thymine
- L. Cytosine
- M. Guanine

D4 The human body's immune system can normally distinguish the body's own cells from other cells because of protein markers, called the major histocompatibility complex (MHC), on the surface of the cells. Each person's cells have a unique MHC. In some circumstances the immune system recognises a person's own cells as being foreign, and attacks them. The attack on a person's own cells could be due to

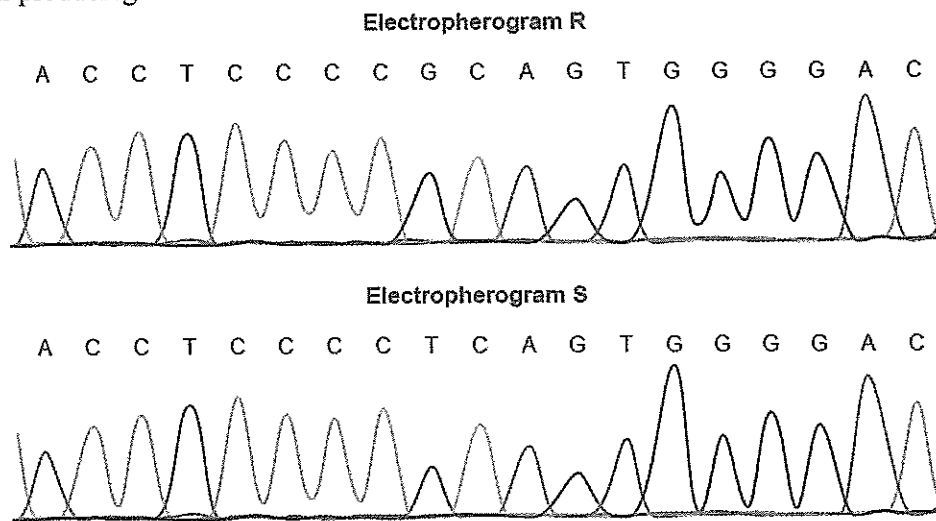
- J. a change in the shape of the MHC gene(s).
- K. an increase in the number of MHC protein markers on the surface of the cells.
- L. a change in the nucleotide sequence of the MHC gene(s).
- M. an increase in temperature that causes the MHC protein markers to denature.

[2010 Q4]

D10 Scientists, working with farmers and other members of the agricultural industry, are studying epigenetic markers to determine whether they provide a clue to the likely productivity of livestock and crops. One investigation involved the dairy industry, one dairy farm, and scientists from several universities. The dairy cows in one herd were tested to determine whether epigenetic changes were responsible for a sudden decrease in the volume of milk produced by some of these cows. All the cows in this herd received the same type of food during the investigation. The scientists found different DNA methylation patterns between high-milk-producing cows and low-milk-producing cows, including in the genes required for milk production.

- (a) Explain how methylation of the genes that are required for the production of milk could affect milk production.

The scientists who were investigating milk production by dairy cows needed to identify the genes that had different DNA methylation patterns, in order to test the validity of their conclusions. The diagrams below show electropherograms of a segment of a dairy cow gene that is required for milk production. Mutations in this gene have been linked to the ability of individual cows to produce milk. Electropherogram R shows the nucleotide sequence of a gene in a high-milk-producing cow and electropherogram S shows the nucleotide sequence of the same gene in a low-milk-producing cow.



Source: Adapted from Marzooq, A AL 2015, 'Discovery of novel DNA variants in Jordanian population by re-genotyping affymetrix DMET arrays data using DNA sequencing', *Molecular biology*, vol. 4, no. 3, figure 4.73 (CC BY 4.0)

- (b) Identify the technique that could be used to determine the order of the nucleotide bases in a gene.
- (c) (i) On electropherogram S, circle the base that is different from the base in the same position on electropherogram R.
- (iii) Explain how this difference in the DNA sequence could alter the function of the gene product.

[Adapted, 2019 Q16]

D11 Beckwith-Wiedemann syndrome (BWS) is a rare disorder in which individuals have abnormal growth and an increased risk of childhood cancer. Cyclin-dependent kinase inhibitor 1C (CDKN1C) is a protein that inhibits cell division. In human beings, this protein is coded by the CDKN1C gene. In some patients with BWS, there is more DNA methylation of the CDKN1C gene than there is in individuals who do not have BWS.

- (a) State the name of the DNA nucleotide that is most often methylated.
- (b) State the effect of increased DNA methylation of the CDKN1C gene on its expression.
- (c) Explain how altering the expression of the CDKN1C gene could lead to cancer.

[2018 Q16]

D14 In normal human brain cells the PRNP gene found on chromosome 20 codes for the membrane protein, PrPc. The most likely function of the PrPc protein is to enable the transport of copper ions with the concentration gradient into cells.

- Describe how a membrane protein could enable the transport of copper ions with the concentration gradient into a cell.
- Genetic Creutzfeldt-Jacob disease (CJD) is caused by an abnormal PrPc protein. Genetic CJD occurs when the abnormal PrPc protein is formed because of a mutation in the PRNP gene. Genetic CJD can be diagnosed by identifying the mutation on chromosome 20.

State one way that chromosomes can be distinguished from each other.

- State two factors that can increase the rate of mutation.
- The mutation that most commonly causes genetic CJD occurs in DNA base triplet 200 of the PRNP gene as shown below:

DNA base triplet number	199	200	201
Normal PRNP gene	TGG	CTC	CAA
Mutated PRNP gene	TGG	TTC	CAA

mRNA codon	Amino acid
ACA, ACC, ACG, ACU	Threonine
AAA, AAG	Lysine
CAA, CAG	Glutamine
CUA, CUC, CUG, CUU	Leucine
GAA, GAG	Glutamic Acid
UGG	Tryptophan
UUC, UUU	Phenylalanine
UUG, UUA	Leucine

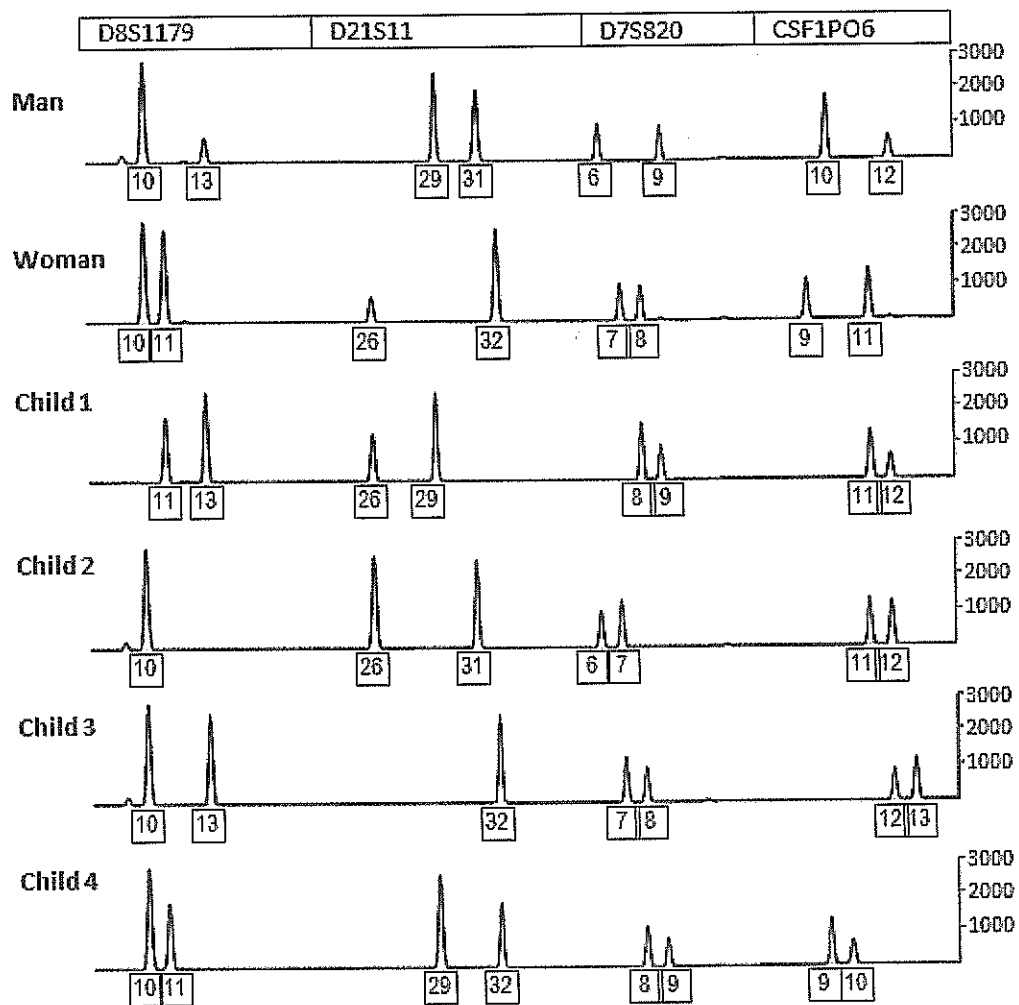
Using the information in the table above, state the *amino acid* substitution that will occur because of the mutation in DNA base triplet 200:

From: \_\_\_\_\_ to: \_\_\_\_\_

- The abnormal PrPc protein has an exposed chain of amino acids which the normal protein does not have.
  - State how a change in the amino acid sequence of the PrPc protein could expose this chain of amino acids.
  - State how this exposed chain of exposed amino acids may prevent copper ion transport into a cell.

[2007 Q26]

- E4 Refer to the following diagram, which shows six DNA profiles: the DNA of a man, a woman, and the woman's four children.



Which child is *not* the biological offspring of the man?

- J. Child 1.
- K. Child 2.
- L. Child 3.
- M. Child 4.

[Adapted, 2010 Q5]

- E5 Genes in organisms can be manipulated by human beings in order to produce useful substances. One example of a useful substance produced in this way is the human protein insulin, which is used to treat the disease diabetes.

Which one of the following procedures most accurately describes how human insulin could be produced and used to treat the disease diabetes?

- J. Take insulin from a human being and grow it in *E. coli* bacteria. Use the insulin produced to treat human diabetes.
- K. Take insulin genes from a human being and place them in *E. coli* bacteria. Use the insulin produced to treat human diabetes.
- L. Take insulin genes from *E. coli* bacteria and place them in a human being, enabling the human being to produce insulin.
- M. Take insulin from *E. coli* bacteria and place it in a human being, enabling the human being to produce insulin.

[2004 Q16]

- E6 *Bacillus thuringiensis* (Bt) is a bacterium that synthesises crystal-like proteins (Cry proteins). Cry proteins bind to specific receptors on the intestinal lining of specific groups of insects, rupturing the cells and killing the insects within a few days. Cotton plants do not naturally synthesise Cry proteins. Biotechnology is used to produce cotton plants that do synthesise Cry proteins.

Which one of the following processes could be used to produce a cotton plant with the potential to synthesise Cry proteins in *all* its cells?

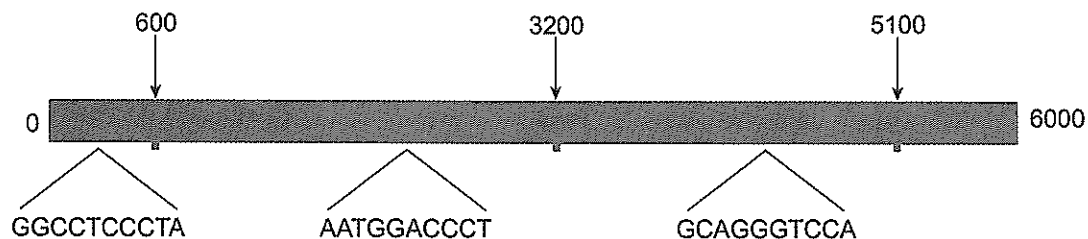
- J. Using a bacterial a bacterial vector to transfer Cry proteins into the cells of a cotton plant.
- K. Transferring the genes for Cry proteins into the cells of a cotton plant.
- L. Using microinjection to transfer Cry proteins into the fertilised egg cell of a cotton plant.
- M. Transferring the genes for Cry proteins into the fertilised egg cell of a cotton plant.

[2009 Q16]

- E7 A DNA molecule that was 6000 bases long was cut with a restriction enzyme. The restriction enzyme cut the DNA at three sites:

- Immediately after the base at position number 600
- Immediately after the base at position number 3200
- Immediately after the base at position number 5100.

Refer to the following diagram, which shows a single strand of the DNA molecule and the base sequences of three regions within the strand:



After the DNA molecule was cut, the resulting fragments were separated by gel electrophoresis and then blotted onto a membrane. A labelled probe with the sequence GGACCCT was then added to the membrane.

The probe will bind to fragment that has

- J. 600 bases.
- K. 1900 bases.
- L. 2600 bases.
- M. 5100 bases.

[2018 Q4].

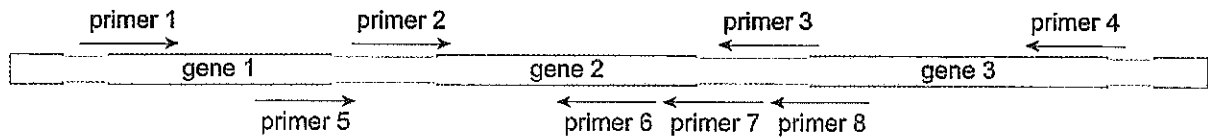
- E8 In the polymerase chain reaction (PCR), a solution containing DNA and an enzyme is repeatedly heated and cooled. The solution is heated to

- J. provide activation energy for the enzyme.
- K. denature the enzyme.
- L. break the weak bonds in the sugar phosphate backbone of DNA.
- M. break the weak bonds between the bases in DNA.

[2003 Q3]



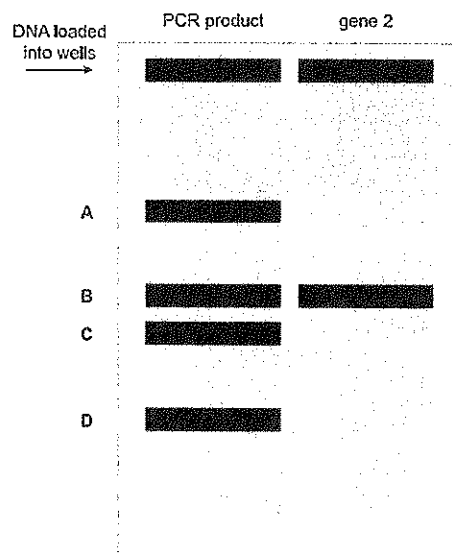
- E13 The following diagram shows a section of DNA that contains three genes: gene 1, gene 2, and gene 3. Primers 1 to 8 are shown as arrows positioned at the location to which each primer will bind and indicating the direction of synthesis.



- (a) State which *two* primers would be used to amplify the smallest length of DNA that contains the whole of gene 2.
- (b) Describe the polymerase chain reaction (PCR), including the roles of primers, free nucleotides, heat-resistant enzymes, heating, and cooling.

The chromosome known to contain gene 2 was cut into smaller fragments using restriction enzymes. Then PCR was used with a pair of primers appropriate for amplifying gene 2. The PCR product was then analysed using gel electrophoresis. Another DNA fragment, which was known to contain only gene 2, was also run on the gel.

The following diagram shows the results of the gel electrophoresis.



- (c) (i) Identify the evidence in the results of the gel electrophoresis that indicates that the PCR product contains DNA fragments of different sizes.
- (ii) State *one* reason why the PCR produced DNA fragments of different sizes.
- (iii) Select the band from the results of the gel electrophoresis that contains *only* the shortest DNA fragment. A  B  C  D
- (d) State why it was necessary to run the DNA fragment known to contain only gene 2 together with the PCR product.

[2020 Q17]

- E14 It is possible to genetically modify an animal by adding copies of a particular gene to the nucleus of a fertilised egg cell. If the gene is incorporated in an appropriate position on a chromosome, it can be successfully expressed.

- (a) State one function of a gene.
- (b) Name one method that could be used in the laboratory to produce multiple copies of a particular gene.
- (c) Name one method that could be used to transfer a gene to the nucleus of a fertilised egg cell.
- (d) Explain why it is necessary to add copies of a particular gene to a fertilised egg cell, rather than to a cell of an adult animal, in order to genetically modify all of the animal's cells.

[2014 Q26]

E19 Golden rice is genetically modified rice that is able to synthesise beta-carotene, which the human body uses to make vitamin A. For rice to synthesise beta-carotene, three new genes are required: two from daffodils and one from a species of bacterium. Beta-carotene gives daffodils their golden colour and is the reason why this genetically modified rice is golden.

- (a) Describe the main steps needed to incorporate in the DNA of rice one of the two genes that have been isolated from daffodils.
- (b) An attempt was made to produce golden rice. To determine whether or not DNA from the daffodils and the bacterium had been successfully incorporated in the DNA of the rice, scientists used PCR and automated electrophoresis to produce DNA profiles. Refer to the following DNA profile of the original strain of rice, three strains of genetically modified golden rice, and the species of daffodil and bacterium used to incorporate beta-carotene genes in the rice:

original strain of rice	strain 1 of golden rice	strain 2 of golden rice	strain 3 of golden rice	daffodil	bacterium

- (i) Identify and explain which one of the strains of golden rice has successfully incorporated DNA from both the daffodil and the bacterium.
- (ii) Describe how automated electrophoresis is used to create a DNA profile.

[Adapted, 2011 Q32]

E20 Progeria is a syndrome that results in premature ageing. The syndrome arises from a single point mutation in the gene LMNA. The resulting abnormal protein is called 'progerin' and it weakens the nuclear membrane. Without the normal form of the protein coded for by the LMNA gene, the ability of the cell to divide is limited.

- (a) Another treatment for progeria uses biotechnology. Describe how the CRISPR technique could be used to treat people who have progeria.
- (b) State which treatment you think would be of most benefit to sufferers of progeria — anticancer drug or CRISPR technique. Justify your answer.

[Adapted, 2018 Sample Exam 17 (b), (c)]

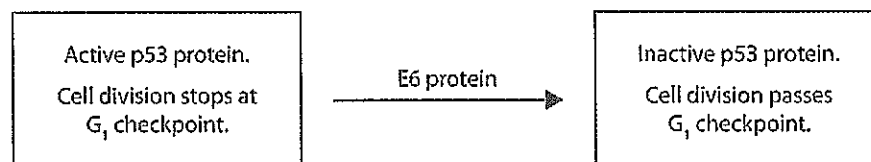
- E21 Scientists have discovered that an enzyme called GalNAc-T6 is *absent* in healthy colon tissue, but *present* in colon cancer cells. The research team used CRISPR/Cas9 on colon cancer cells to inactivate (knockout) the *GalNAc-T6* gene and produce cells that could not synthesise GalNAc-T6. These cells showed more normal growth than cells with an active *GalNAc-T6* gene.
- Explain why it is necessary to know part of the base sequence of the *GalNAc-T6* gene in order to use CRISPR/Cas9 to inactivate the gene.
  - Describe *one* consequence of the guide RNA that is attached to the Cas9 protein being too short.
  - State *one* function of the Cas9 protein that is used to edit the *GalNAc-T6* gene.
- [2018 Q21]

- E22 In February 2013 a skeleton was identified ‘beyond reasonable doubt’ as that of King Richard III of England, who died in 1485. The evidence used to identify the skeleton included mitochondrial DNA (mtDNA), a small circular DNA molecule found in each of a cell’s mitochondria. It is inherited only from the mother. The mtDNA in the skeleton was compared with that of a living person known to share a common ancestor with King Richard III and was found to have significant similarities. Normally, samples of DNA from the nucleus of the cell (nuclear DNA) are collected. These samples then undergo the polymerase chain reaction (PCR) before further analysis.

Explain why examining mtDNA rather than nuclear DNA from a sample of tissue may reduce the need for PCR. [2013 Q27]

- E23 Human papilloma virus (HPV) is known to cause cervical cancer. HPV contains the *E6* gene, which codes for a protein called E6. The E6 protein inactivates the human p53 protein, which is coded for by the tumour-suppressor gene called *p53*. Women who have the inactive p53 protein are likely to develop cervical cancer. In 2019, it was reported that researchers had developed a potential treatment for cervical cancer using the CRISPR technique. One target for the treatment is the *E6* gene.

The effect of the E6 protein on the p53 protein is summarised in the diagram below.



- Explain how changing the sequence of amino acids of the p53 protein may affect its function.
- Explain why a unique base sequence of part of the *E6* gene must be known if the CRISPR technique is to succeed.

It has been proposed that genetic information should be collected from all women in Australia in order to better understand cervical cancer and hence develop more effective treatments.

- Explain *one* ethical issue and *one* economic issue related to the proposed collection of this genetic information.
    - Ethical issue:
    - Economic issue:
- [2021 Q16]

- E24 Transgenic sheep have been developed that produce a human protein used to treat people suffering from the disease cystic fibrosis. This protein can be easily extracted from the sheep’s milk.

- Explain why the gene for the human protein must be transferred into the sheep at the zygote stage of the sheep’s development rather than into the cells of an adult sheep.
- State one *ethical* reason *against* the production of transgenic organisms.

[Adapted, 2007 Q30]

Suggested

**ANSWERS**

## 9.2 DNA AND PROTEINS

### DNA Structure, Function and Replication

A1 L    A2 K    A3 J    A4 J    A5 L    A6 M  
A7 M    A8 L    A9 M    A10 M    A11 L    A12 M

- A13 (a) Cytoplasm OR Attached to membrane  
(b) Each daughter cells needs an identical /complete copy of the parent's DNA. DNA replication produces two identical copies of the DNA.  
(c) Semi-conservative means "half old – half new". Diagram shows original strands (old) in yellow and new strands in grey.
- A14 (a) A  
(b) Prokaryotic cells have circular chromosomes whereas eukaryotes have linear. Eukaryotic DNA is coiled around histones whereas prokaryotic have no histones. Eukaryotic cells have introns (non-coding) DNA whereas most prokaryotes have no introns.  
(c) Nucleus  
(d) Nucleotide composition and pairing. The composition of nucleotide bases allows for two hydrogen bonds to form between A-T and three between C-G bringing about the specific base pairing rule. During replication, this allows free floating nucleotides to form complementary strand with the exposed bases of the template strand of DNA. OR Weak hydrogen bonds between strands; During DNA replication the weak hydrogen bonds allow the DNA molecule to be unzipped, exposing bases for the formation of two new strands complementary to the template strand. The collective forces created by hydrogen bonds provide the two newly formed double helices with stability and allows for the formation of the double-helix structure.  
(e) A description of semi conservative DNA replication in eukaryotes. Each strand acts as a template for the other strand, so that each new molecule of DNA contains one old and one new strand of DNA.
- A15 Semiconservative DNA replication produces two identical DNA molecules, each molecule will contain one original strand and one newly synthesised strand. When DNMTs are not present, the newly synthesised strands will not be methylated.
- A16 Structure: the two strands of DNA are anti-parallel. One strand is 5' to 3' and the other 3' to 5'. This enables the two strands of DNA to be connected to each other through complementary base pairing.  
Transcription: the 5' to 3' direction of the DNA of a gene encodes the sequence to produce the corresponding gene product. Therefore, to produce a copy of the gene, in the form of messenger RNA, during transcription, the 3' to 5' strand will be used as the template. The messenger RNA will also be 5' to 3'.

### Genes and Protein Synthesis

B1 L    B2 M    B3 M    B4 L    B5 J    B6 L  
B7 M    B8 L    B9 K    B10 J    B11 M    B12 L  
B13 L    B14 L    B15 M    B16 K    B17 J    B18 K  
B19 M

- B20 (a) Translation  
(b) (i) CTA  
(ii) The enzyme's active site has complementary shape to a specific amino acid. Different amino acids/tRNA have different shapes

- (c) rRNA (Ribosomal RNA) combines with proteins make up a ribosome. Ribosomes are the site of protein sythesis and catalyze the assembly of amino acids into protein chains.
- (d) ATP provides energy for synthesis reactions
- B21 (a) (i) Nucleus  
(ii) Prokaryotic cells do not have a nucleus (or membrane bound organelles), this process occurs in their cytoplasm.
- (b) In eukaryotic cells, mRNA contains introns and exons. Only exons are coding, so introns are non-coding and therefore need to be removed before translation.
- B22 (a) Any one of: T replaced by U in RNA, single strand base sequence in RNA, deoxyribose replaced by ribose in RNA, RNA is a smaller molecule.
- (b) U C C G
- (c) MicroRNA or miRNA regulates/prevents gene expression. Or prevents a protein being made from mRNA.
- (d) miRNA prevents gene expression. During translation mRNA binds to tRNA. These sites are now blocked by miRNA preventing translation.
- B23 (a) Translation
- (b) DNA coding strands (gene) corresponds to the codons/ nucleotide sequence on mRNA which codes for the amino acid sequence required to build a protein.
- (c) mRNA – carries copy of DNA code from nucleus to cytoplasm  
tRNA – brings specific/correct amino acid to ribosome in cytoplasm.
- (d) (i) immature mRNA contains introns/ non-coding regions of DNA  
(ii) Introns are removed from the immature mRNA/ pre-mRNA and exons are joined together.  
(iii) Eukaryotic DNA contian both introns and exons. Introns are non-coding regions of a gene where as exons are coding regions that are translated/ expressed into a protein.
- B24 (a)  $4 \times 4 \times 4 \times 4 = 256$
- (b) mRNA transcribed one base at a time not in “blocks” of 3 or 4; number of nucleotides that make up a codon has little to do with transcription (but it does in translation)
- (c) Four bases would be required in the anticodon
- (d) e.g. “New” proteins may – be more persistent in the environment and will not be easily broken down; – produce “new” life forms that are undesirable/harmful; – have negative side-effects if in contact with humans/other life; – be unethical
- B25 (a) 103050
- (b) Describe translation: The mRNA binds to the ribosome, tRNAs with an anticodon complementary to the codon on the mRNA, carry a {specific} amino acid to the ribosome, amino acids are then joined together by a peptide bond to create a polypeptide chain.
- (c) Ionising radiation such as UV, X-rays OR viruses
- (d) Introns are removed to produce an mRNA molecule composed only of exons
- (e) If there are additional exons, there would be more codons and this would lead to additional amino acids in the polypeptide/protein. With a longer polypeptide, this will likely change the folding or change the shape of the polypeptide. As the function of a protein is determined by its shape, this new form of titin may not function normally or as effectively.

## Protein Structure and Function

C1	L	C2	L	C3	M	C4	L	C5	M	C6	M	C7	L
C8	K	C9	M	C10	L	C11	M	C12	K	C13	M	C14	J

C15 K    C16 J    C17 K    C18 L    C19 J    C20 L    C21 M  
 C22 L    C23 L    C24 J

- C25 (a) The induced fit puts a strain on bonds of substrate (acetylcholine), reducing the energy required to break the bonds.  
 (b) DIFP either blocks or changes the shape of the active site of AChE. Acetylcholine can no longer bind therefore is not broken down.
- C26 (a) Maintains cell structure or shape *or* any other function of cytoskeleton  
 (b) Decreased ROCK results in decreased LIMK, which results in increased cofilin (decreased p-cofilin). This enables the actin filaments to remain dynamic and able to change.  
 (c) The enzyme ROCK has the only active site that LIMK can bind to because it has an active site with a shape that is complementary to the shape of the LIMK molecule. Other appropriate answers may include an explanation that other enzymes do not have that complementary active site.  
 (d) Elevated levels of p-cofilin result in actin filaments that are neither dynamic nor able to change. This reduces the growth of new dendritic spines and/or the enlargement of existing ones and these are required for learning and memory. The answer must reflect the relationship between p-cofilin and memory: effect of p-cofilin on filaments → effect on dendritic spines → link to memory and learning  
 (e) Describe either competitive or non-competitive inhibition. For example, competitive: Fasudil has a shape that is complementary to the active site on ROCK. It binds to the active site on ROCK, preventing the substrate from binding or Non-competitive: Fasudil binds to site other than active site changing or altering the shape of the active site, preventing the substrate from binding
- C27 (a) Procaspase  
 (b) To protect the cell from cell death or stop healthy cells from dying.  
 (c) The active site on caspase 8 has a shape that is complementary to the shape of the substrate, procaspase 3  
 When these two molecules, an enzyme/substrate join together via induced fit, there is a strain put on the bonds of procaspase 3, forming caspase 3 or the use of the enzyme reduces the activation energy required to produce caspase 3.  
 (d) An answer relating to either a competitive inhibitor or non-competitive inhibitor.  
 Competitive inhibition: An inhibitor with a similar shape to substrate (procaspase 3) (binds to and) blocks the active site of enzyme (caspase 8), so that substrate (procaspase 3) cannot join to it or non-competitive inhibition.  
 A non-competitive inhibitor binds to a place on the enzyme (caspase 8) other than the active site, changing the shape of the active site, and preventing the binding of the substrate (procaspase 3) to the active site of the enzyme (caspase 8).
- C28 (a) Enzyme is denatured at temperatures above 45°C  
 (b) Invertase lowers the activation energy for the reaction  
 (c) The inhibitor may compete with substrate for a position at the active site of the enzyme. The higher the substrate concentration the greater is the likelihood that the substrate will bind to the enzyme.
- C29 (a) Could provide an answer referring to iDNMTs as either competitive inhibitors or non-competitive inhibitors.  
 Competitive Inhibitors- are complementary to the shape of the active site and hence will bind to the active site which will stop the substrate binding and so prevent the DNMT from catalysing methylation  
 Non-competitive inhibitors - will bind to an alternative binding site, this changes the shape of the DNMT protein's active site stopping the substrate binding and so preventing the DNMT from catalysing methylation.

- (b) DNMTs could inhibit the methylation of other genes (besides TSGs) leading to a range of unintended effects.

C30 Various possible answers: Each step loses some energy as heat, preventing excessive heat production so that enzymes do not denature  
Some steps produce intermediate compounds, which can then be used in other metabolic pathways.  
Specific enzymes are required at each step, this allows for regulation of the metabolic pathway.

### Phenotypic Expression and Mutations

D1 M D2 L D3 L D4 L D5 K D6 K  
D7 M D8 K

- D9 (a) cytosine  
(b) Increased methylation of a TSG will result in decreased expression of the TSG. This will lead to uncontrolled cell division.

- D10 (a) Methylation reduces gene expression or switches gene off or prevents transcription.  
(b) DNA or gene sequencing, or electrophoresis  
(c) (i) Circle T (near the centre) or the corresponding peak.  
(ii) This would change one codon (mRNA sequence) and this may result in a change to one amino acid in the polypeptide. The gene product (protein) could have a different overall shape, which could alter its (protein) function

- D11 (a) Cytosine  
(b) Reduced expression/silenced/switched off/ transcription prevented.  
(c) The protein produced by the *CDKN1C* gene inhibits cell division. Reducing the expression of the *CDKN1C* gene leads to less protein. This could result in uncontrolled cell division, which is a cause of cancer.

- D12 (a) One strand is maintained/old – it is conserved and used as a template; the other half of molecule is copied and is new  
(b) Transcription: DNA unwinds/unzips to expose the bases of that contain the specific sequence (gene) for telomerase; exposed bases are paired with nucleotides that form mRNA (according to base pairing rules)  
(c) Preventing the regulation of telomerase production will cause *overproduction* of telomerase  
Changes in the telomerase gene/increase in telomerase will lead to increase in cell division – cancer is uncontrolled cell division  
(d) ONE of ionising radiation, mutagenic chemicals, viruses.  
(e) Competitive inhibitors have a complementary shape to the active site of the enzyme (or a similar to the substrate); they prevent the substrate from occupying the active site (by blocking it) OR  
Non-competitive inhibitors bind to the enzyme causing a change in shape of the active site; substrate will no longer have complementary shape to the (changed) active site and will not bind properly  
(f) One example needed. Possibilities include:  
Only the rich can afford to buy it – e.g. leading to division of class in society; greater strain on health system/societal resources; fewer people retiring – increased unemployment; overpopulation leading to a strain on resources

- D13 (a) If genes are missing or switched off, gene products/proteins not produced that are required in meiosis / gamete production.  
(b) DNA methylation of cytosine or could say acetylation or histone modification  
Prevents transcription / gene expression / prevents access to the DNA/gene.



- D14 (a) Provides a channel/binds to the ion  
 (b) Different size/shape/genes/banding patterns  
 (c) TWO of ionising radiation, mutagenic chemicals, viruses.  
 (d) Glutamic acid to lysine  
 (e) (i) Changes the way in which the protein folds  
 (ii) May block the channel or affect the binding

### Biotechnology

E1 M E2 J E3 L E4 L E5 K E6 M  
 E7 K E8 M E9 M E10 L

- E11 (a) Separate DNA strands to expose bases by heating; add a probe (single stranded DNA or RNA) which is complementary to gene of interest – mark the probe (e.g. radioactive, fluorescent dye)  
 (b) Increases chances of desired copy being incorporated with host DNA OR  
 Not all copies will incorporate successfully  
 (c) Mitosis OR Mitotic  
 (d) So that consumers can make an informed choice/they are aware of what they are using  
 (e) Two parents means sexual reproduction which relies on meiosis to generate gametes. Genetic variation results from – random fertilisation, crossing over, independent assortment  
 (f) High temperatures will cause human polymerase enzyme to denature (irreparable damage to the protein structure) and hence the polymerase would not function.
- E12 (a) Protein  
 (b) (i) Complementary primers bind to section of DNA (to be amplified) which provides starting point for synthesis of DNA or identify where the DNA polymerase will bind and prevents the DNA strands from re-joining.  
 (ii) DNA synthesis or create new DNA molecule by joining of free nucleotides / bases or joins sugar-phosphate backbone together.  
 (c) The sample is initially heated to separate the DNA strands, it is then cooled to allow the primers to bind, and then heated again to the optimum temperature for the DNA polymerase to synthesise the new DNA or alternatively could indicated that repeating allows DNA to be multiplied/amplified/large numbers of copies to be made.  
 (d) No comparison / cannot compare since it is extinct or there is no live specimen. Or alternatively could say that the preserved specimen may have been degraded / contaminated which would affect the comparison.
- E13 (a) 2, 7  
 (b) First, heat the template DNA to 'melt' it (separate the strands). Primers indicate the 'start' and the 'end'. Cool to allow two primers to bind (anneal), one to each strand. Free nucleotides are the 'building blocks' of the new DNA. Heat to allow the free nucleotides to bind to the exposed bases, in the presence of the heat resistant (Taq) DNA polymerase. Enzymes need to be heat resistant to tolerate the heating cycle.  
 (c) (i) There are four bands (A, B, C, D), indicating fragments of different sizes .  
 (ii) The primers bound to the DNA at different sites.  
 (iii) B and D.  
 (d) To determine which band contained gene 2.
- E14 (a) Code for polypeptide or RNA molecule  
 (b) Polymerase Chain Reaction  
 (c) Bacterial plasmids OR Viruses OR Microinjection

- (d) All of the animal's body cells come from the fertilised egg cell, and are genetically identical to it. If the gene is added to the cell of an adult animal, the only cells that will contain a copy of the gene will be those that arise from the division of that cell.

- E15 (a) Karen is homozygous at loci vWA. Both paternal and maternal alleles are the same length/ had 15 Short Tandem Repeats (STRs) at the vWA loci.

(b)

	Karen
D5S818	9, 13
vWA	15, 15
FGA	19, 20

- (c) (i) STRs are regions of DNA that contain the same repeating nucleotide sequence and can be found at different loci on different chromosomes. The number of STRs varies between individuals and can be determined from the size of the STR fragment collected in electrophoresis. Offspring have common STR repeats to their parents as they inherit one set of chromosomes from the mother and one from the father. Thus the number of STR repeats/ allele values of offspring can be compared to others determine family lineages.
- (ii) Karen and Ben
- (iii) Karen
- (d) (i) High temperature is required to separate strands of DNA.
- (ii) DNA has some structure in all species.
- (iii) Primer identifies the start point for the copying of DNA/Stops DNA strands joining together

- E16 (a) Probe would be a short section of single stranded DNA or RNA that has a base sequence that is complementary to part of the gene. It would either be radioactively labelled or dyed.
- (b) Cut plasmid with same restriction enzyme used to cut the gene. Use an enzyme (ligase) to join gene and plasmid.
- (c) All (20) amino acids are needed to make the full range of proteins in bacteria. Depletion of two amino acids results in inability to make proteins necessary for bacterial cell function.
- (d) e.g. Animal rights – goats could be harmed during GE process/collection of 'silk milk'.

- E17 (a) Made of DNA or RNA; single-stranded; labelled; complementary to the GHSP26 gene
- (b) The gene for the gene for Bt toxin will have a different base sequence from the gene for GHSP26. Therefore, the probe will not bind to it.
- (c) Remove plasmids from bacteria. Cut with a restriction enzyme. Cut the gene for GHSP26 from the donor cell using the same restriction enzyme to ensure complementary sticky ends. Mix the gene with plasmids and use ligase to join together. Insert plasmids into bacterial cells and use them to 'infect' the cotton plants.
- (d) The genetic code is universal, so it will code for the same protein in all cells.
- (e) mRNA binds to ribosome, one codon read at a time, tRNA with complementary bases delivers a specific amino acid, joining amino acids to form polypeptide

- E18 DNA sequencing could be used to identify different species by using specific genetic markers- probably would need to use a number to ensure the accuracy of the information. Sequence DNA from each fish species in question to determine the base sequence and then confirm species by comparison to a known sequence- as each species would have its own specific sequence for each gene used.
- Discuss: Various answers possible- for example could address: should DNA sequences be collected and kept? Who controls this information, for what purpose? Not everyone may be able to access this DNA information- equity issues. Accessing the information may cost money- again will it mean not everyone can access? Could it be used to save money for criminal

investigations? Everyone's DNA on a database, screen at crime scene- lead to suspects more quickly? Concerns for false readings etc..

- E19 (a) EITHER  
 Same restriction enzyme used to splice a gene into a plasmid (during the isolation of the gene) would need to be used to then remove it for preparation of vector for insertion into the rice OR  
 Preparation of the gene for insertion e.g. copies of gene need to be made – PCR OR cut out the DNA from plasmid and attach to a gold particle OR put the gene into a viral vector using the same restriction enzyme OR use of agrobacterium and the Ti plasmid  
**THEN**  
 Put the gene into the rice cells for incorporation into the rice genome via a vector: plasmid, virus, gene gun OR microinjection (with description) OR electroporation
- (b) (i) Strain 2 of Golden Rice  
 EVIDENCE: Strain 2 of Golden Rice has incorporated DNA from both daffodils and bacteria is presence of DNA fragments that correspond to some fragments from both the daffodil and bacteria
- (ii) Florescent tagged primers are used in PCR to amplify STR regions of DNA in a sample. This is placed into the capillary tube of a automated electrophoresis machine that separates STR fragment based on size. DNA is negatively charged and when a current is applied it travels towards a positive electrode. The smaller fragments migrate faster and the florescent dye of each STR fragment is detected by a laser. Produces an electropherogram with allele values for each STR loci. The results of electrophoresis can be used to construct a DNA profile.
- E20 (a) CRISPR could be used to fix the point mutation in the LMNA gene. This could occur if the correct guide RNA molecule is made, so that the CAS protein can recognise the mutation and correct it by replacing the nucleotide base with the correct one.
- (b) Various answers possible: CRISPR- permanent change, reduce costs of medications, doctor's visits, no side effects compared to the use of anti-drugs etc.
- E21 (a) The Cas9 protein needs to be 'programmed' with guide RNA to enable it to locate the *GalNAc-T6* gene. The guide RNA must have a base sequence that is complementary to part of *GalNAc-T6* gene.
- (b) If the guide RNA is too short, then the Cas9 protein might bind to other sites and a consequence to "other" DNA or could cause an effect not only at the intended site.
- (c) It cuts the DNA at a specific position
- E22 Each cell has many mitochondria and therefore many copies of mtDNA. Less need for PCR to amplify mtDNA
- E23 (a) Alters the folding/coiling (secondary structure) of the polypeptide, which alters the 3D shape (tertiary structure) of the polypeptide, inhibits function of p53 / inactivates p53 / inability of p53 to function / p53 cannot function normally / will not stop cell division.
- (b) Design a guide RNA that is complementary to the target gene, to identify where the cas9 endonuclease protein must cut.
- (c) (i) Ethical issue with an elaboration e.g. An invasion of privacy that the individual may not want known or are concerned information may be used inappropriately
- (ii) Economic issue with an elaboration e.g. Large costs/funding which would need to be paid by the taxpayer / Government / indirectly by consumers.
- E24 (a) If placed in the zygote *every cell* will have a copy of the gene. If placed in the adult sheep *only some cells* will have a copy of the gene
- (b) e.g. May cause pain to the animal/animal did not consent to the process/tampering with natural processes.  
 Could affect the functioning of cells/environmental impact if gene escapes into wild populations