Name: _____

Biology

Question and Answer Book

Unit 3, Area of Study 1 - Analysis and evaluation of contemporary bioethical issues

- Reading time is **5 minutes**
- Writing time is **55 minutes**

Materials supplied

- Question and Answer Book of 8 pages

Instructions

- Follow the instructions outlined at the beginning of Section A.

Students are not permitted to bring mobile phones and/or any unauthorised electronic devices into the examination room.

Contents pages

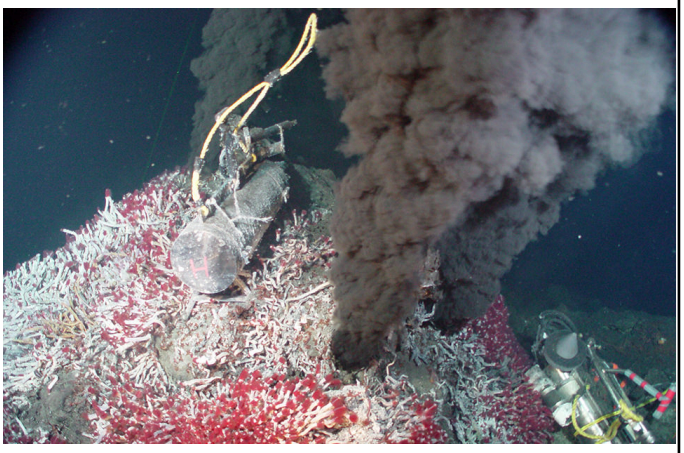
Section B

Instructions

- Answer all questions in the space provided.
- Write your responses in English.
- Unless otherwise indicated, the diagrams in this book are **not** drawn to scale.

Question 1 (8 marks)

Meet Luca, the Ancestor of All Living Things



Scientists have unveiled a detailed genetic profile of LUCA, the Last Universal Common Ancestor, shedding light on the origins of all living things on Earth. LUCA, estimated to have lived four billion years ago, was a single-cell, bacterium-like organism. Research led by William F. Martin of Heinrich Heine University in Düsseldorf, Germany, analyzed six million protein-coding genes from bacteria and archaea, identifying 355 genes likely present in LUCA. The degeneracy of DNA triplets is crucial for understanding the evolutionary relationships and origins of living organisms. Despite the diversity of DNA sequences, LUCA's genetic makeup provides a common ancestral thread among bacteria, archaea and eukaryotic cells.

The genes suggested an organism adapted to deep-sea vent conditions, challenging rival theories proposing extreme environments like volcanic flanks or Darwin's "warm little pond." While some argue LUCA was "half alive" and close to life's origin, sceptics, including chemist John Sutherland, propose a more sophisticated LUCA and question the deep-sea origin of life, suggesting the possibility of a land-based organism. The controversy highlights ongoing debates over the environment and conditions conducive to life's emergence on Earth.

Source: '*Meet Luca, the Ancestor of All Living Things' by Nicholas Wade. July 25, 2016.*

a. Define the term "degenerate" and explain how this minimises the impact of deleterious mutations.

3 marks

b. Explain how the genome and the proteome are related in an organism. In LUCA or other distant prokaryotes, describe how the number of genes present influences the range of proteins that can be produced.

2 marks

c. Give one example of a type of protein within LUCA that may change in concentration and explain why this is necessary. 3 marks

Question 2 (12 marks)

A new insulin given approval for use in the U.S.

On the 30th of October 1982, the New York Times reported that the U.S. Food and Drug Administration (F.D.A) had approved the use of “Humulin”. The first drug produced using recombinant bacteria technology to be granted approval for human use, in the United States of America. Following the approval of Humulin, other proteins produced through recombinant technologies were also approved including human growth hormones and interferons.

Prior to the availability of synthetic insulin, diabetics used insulin derived from the pancreases of pigs and cattle that were purchased, by laboratories, from slaughterhouses.

Human insulin is a small globular protein containing two peptide chains (A and B). The function of insulin is to bind to membrane-bound receptors on the surface of cells, facilitating the uptake of glucose via intermembrane proteins such as GLUT4. Insulin production in β-cells of the pancreas is increased under certain bodily conditions.


The New York Times

A New Insulin Given Approval For Use in U.S.

F.D.A. Permit Is First for Gene-Splicing Product

REPUBLICANS FOCUS ON TROUBLE SPOTS IN CONGRESS RACES

U.S. SEEKS ACCORD WITH ALLIES ON END OF PIPELINE CURBS

Moscow Says It Is Still Committed To Improving Ties With the U.S.

Spain's New Leader: Young Lion of the Left

Most Office Towers Still Violate City Fire Code

Source: ‘*A new insulin given approval for use in U.S.” by Lawrence K. Altman. October 30, 1982.*

a. Describe the role of DNA ligase in the production of Humulin. 1 mark

b. State insulin's hierarchical level of organisation. Justify your answer. 2 marks

c. In reference to Humulin, outline what would occur at the ribosome to result in the production of Insulin's A chain. 3 marks

d. Explain the importance of antibiotic resistant genes in the production of human insulin using recombinant plasmids. 3 marks

e. Discuss an ethical concern that could be raised from the original source of insulin from animal pancreases. State the ethical principle that has been addressed in your discussion. 3 marks

Question 3 (9 marks)

Tryptophan is the least abundant amino acid in eukaryotic cells and is only coded for by a single codon. Humans need to consume tryptophan in their diets, however *Escherichia coli* are able to synthesise their own. *E. coli* are able to control the production of tryptophan through the use of a repressor molecule and through a process known as attenuation.

a. Compare prokaryotic and eukaryotic gene expression. 3 marks

b. Explain the role of the leader region in *E. coli's* survival. 3 marks

Scientists want to be able to control the amount of tryptophan produced by *E. coli* and are therefore developing a synthetic tryptophan repressor molecule (TrpR). TrpR is made up of two subunits containing six α-helices.

c. Explain what scientists will need to consider when producing this synthetic molecule to ensure it appropriately inhibits tryptophan production. 3 marks

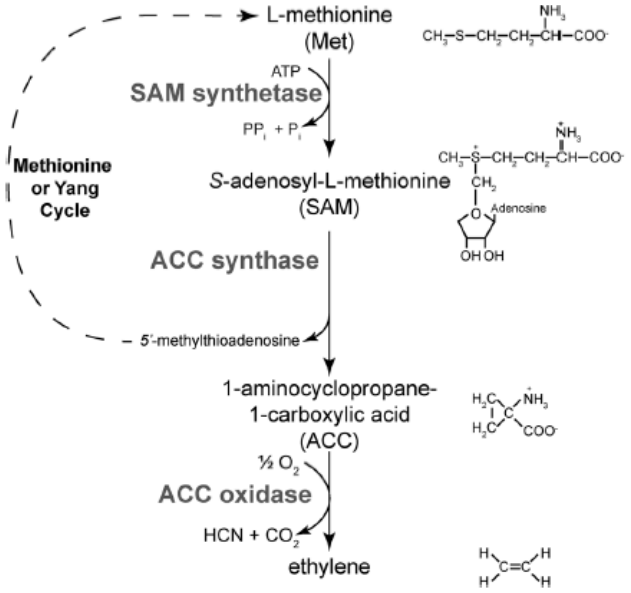
Question 4 (11 marks)

Due to growing populations and high urbanisation, many supermarkets rely on fruit and vegetables that have been grown and harvested internationally. Fresh produce sourced internationally is at risk of "over-ripening" before it is available to consumers. This can result in unsatisfactory products for customers and a direct financial loss for supermarkets, as they are unable to sell their products. Ethylene is a hormone released by plants to stimulate ripening of fruit.

The Flavr Savr tomato designed by Calgene in the early 1990's aims to reduce the proportion of over ripened fruit. Traditionally, companies inserted a synthetic mRNA transcript to decrease the production of ethylene.

However, more recently, biotechnology companies have aimed to reduce the softness of overripe tomatoes by inserting a gene from a different species that alters a key step in the ethylene production pathway.

The pathway for ethylene production is shown below:



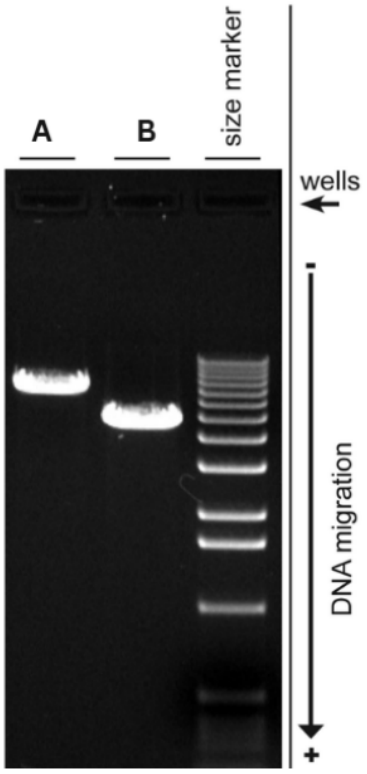
Source: Ufitinema, B., Niyomugabo, J., Hakizimana, M. & Hakiruwizera, E. 2024, 'The potential of nitric oxide in minimising postharvest physiological changes and ethylene production in stored fruit', International Journal of Advanced Research and Reviews, vol. 12, pp. 52–62, doi: 10.54978/ijarr.2023.

a. State if the Flavr Savr tomato produced by Calgene is a transgenic organism. Justify your response.

2 marks

b. Using the information in the flow chart, above, explain how CRISPR-Cas9 technology can be used to insert a gene to reduce the overripening of fruit. 4 marks

A group of researchers wanted to investigate the DNA sequence of the tomatoes containing an additional gene, in comparison to organically grown non-GMO tomatoes. Their findings are displayed below.



Source: Dr d12, 2006, DNAgel4wiki.png [image], Wikimedia Commons, https://commons.wikimedia.org/wiki/File:DNAgel4wiki.png

c. With reference to the information, above, state which of the wells **(A or B)** has the modified gene. Justify your response. 2 marks

d. Describe the function of the CRISPR sequence in bacteria. 3 marks

End of Section A

Unit 3, Area of Study 1 assessment report

General Comments

Overall, students demonstrated a sound foundational knowledge of gene structure and protein synthesis, with particular strength shown in responses requiring definition, such as the term 'degenerate' (Question 1a). Students are to be commended for their ability to articulate a clear link between a point mutation and the potential for a silent outcome.

However, a noticeable area for improvement lies in the accuracy and depth required when applying knowledge to complex biological systems and technologies. Responses often lacked the explicit detail required by the command term 'Explain,' particularly when describing the roles of specific components within a system. For instance, in Question 2d, while many students knew the function of antibiotic resistance genes was related to selection, few were able to fully articulate the multi-step selection process necessary for successful transformation and identification of the recombinant plasmid. Similarly, Question 3b required students to link the function of the leader region in the trp operon to the broader concept of energy conservation, a link that was frequently omitted.

Furthermore, students must ensure they directly address the requirements of comparative command terms. In Question 3a, which asked for a 'Compare' between prokaryotic and eukaryotic gene expression, a significant number of responses focused solely on differences (e.g., RNA processing and co-transcriptional translation), neglecting to identify and articulate the similarities (e.g., both use RNA polymerase and ribosomes to produce a protein product).

Finally, in bioethical analysis (Question 2e), students are reminded of the importance of explicitly linking the ethical concern (e.g., potential for disease transmission from animal-sourced insulin) to the relevant ethical principle (e.g., Non-maleficence), ensuring their discussion includes a clear cause-and-effect relationship to achieve full marks. Students should continue to practice applying the key science skills, especially in justifying claims using specific evidence and connecting complex processes to the overall biological benefit, such as cell survival or energy conservation.

Question 1 (8 marks)
a. Define the term “degenerate” and explain how this minimises the impact of deleterious mutations.

Marks	0	1	2	3	Average
%					

- Degenerate refers to more than one codon coding for the same amino acid
- If a point mutation/addition/deletion/substitution occurs the amino acid coded for may not change or silent mutation occurs
- The primary structure/correct folding/polypeptide chain/shape

Exemplar student response: Degenerate means that more than one codon codes for an amino acid. This minimises negative mutations as if the mutation causes a change in nucleotide it may still code for the same amino acid. The protein that the section of gene produces will stay the same and will remain functional.

b. Explain how the genome and the proteome are related in an organism. In LUCA or other distant prokaryotes, describe how the number of genes present influences the range of proteins that can be produced.

Marks	0	1	2	Average
%				

- Genome provides the genes/information for proteins.
- The number of genes would be the same as number of proteins

Exemplar student response: Genome is the complete set of genes within the organism, proteome is the complete set of proteins within the organism. The genome provides the genetic information for the proteins within the proteome. Genes code for proteins, 355 genes code for 355 proteins.

c. Give one example of a type of protein within LUCA that may change in concentration and explain why this is necessary.

Marks	0	1	2	3	Average
%					

- Contractile
- Increase during cell replication
- Allows cell division

or

- Enzyme/Example of enzyme
- Increase/decrease to increase/decrease the rate of reactions
- To catalyse the reactions for survival/conserve energy

or

- Transport
- Increase to allow for greater movement of nutrients/waste
- To ensure survive

or

- Regulatory protein/Example of regulatory protein
- Can switch gene expression on or off
- Saving energy

Question 2 (12 marks)

a. Describe the role of DNA ligase in the production of Humulin.

Marks	0	1	Average
%			

- Ligase joins the DNA fragments/sugar-phosphate/phosphodiester/phosphate bonds of the recombinant plasmid

No mark for glue
No mark for wrong bonds

Exemplar student response: DNA ligase joins the sugar-phosphate backbone of the gene (insulin gene) and plasmid to create a recombinant plasmid.

b. State insulin's hierarchical level of organisation. Justify your answer.

Marks	0	1	2	Average
%				

- Quaternary
- Insulin is made up of an A chain and a B chain

c. In reference to Humulin, outline what would occur at the ribosome to result in the production of Insulin's A chain.

Marks	0	1	2	3	Average
%					

Translation

- Initiation: mRNA and ribosome combine and a tRNA, carrying the first amino acid, binds to the start codon.
- Elongation: amino acids are brought to the mRNA (codon) by tRNAs (with complementary anticodon) and are added one at a time to a growing polypeptide chain.
- Termination: a stop codon in the mRNA is recognized and the translational apparatus comes apart to release a completed polypeptide.

Exemplar student response: Initiation: mRNA binds to ribosome and start codon (AUG) is recognised by tRNA anticodon (UAC) to deliver methionine and initiate the start of the growing Insulin A polypeptide chain.
Elongation: Ribosome reads mRNA in a 5' to 3' direction and tRNA brings specific amino acids based on complementary anticodons from Insulin A mrna, to the growing polypeptide chain.
Termination: 1 of the 3 stop codons are read and no more amino acids are added to the chain. The ribosome then releases the translation factors to complete the Insulin A chain for use in Humulin manufacturing.

d. Explain the importance of antibiotic resistant genes in the production of human insulin using recombinant plasmids.

Marks	0	1	2	3	Average
%					

Any 3 of
- (AmpR) allows for scientists to determine if the bacteria has been transformed
- (TetR) allows for scientists to determine if the Insulin subunit has been successfully inserted into the plasmid
- Survive exposure to antibiotic plate/selective agent
- Insulin subunit / chain is produced
- Allows for selection of the correct bacteria with Insulin inserted

Don't need to explicitly state Amp and Tet, can describe function
No mark for B-gal (blue screening)

e. Discuss an ethical concern that could be raised from the original source of insulin from animal pancreases. State the ethical principle that has been addressed in your discussion.

Marks	0	1	2	3	Average
%					

Any one of:
- Cause: Animal pancreases may have diseases
- Effect: resulting in sickness in people using insulin
- Non-maleficence

Or
- Cause: Harming animals is unethical/against people's beliefs
- Effect: People won't want treatment
- Non-maleficence, respect, consequences-based

Ethical principle must be relevant to the ethical concern.

Question 3 (9 marks)
a. Compare prokaryotic and eukaryotic gene expression.

Marks	0	1	2	3	Average
%					

Similarities:
- Both undergo transcription/translation producing mRNA
- Both processes result in the production of a protein
- Both involve RNA polymerase and ribosomes

Differences:
- In eukaryotes RNA processing occurs whereas in prokaryotes there is no RNA processing
- In eukaryotes introns are spliced out whereas prokaryotes have exons only
- In prokaryotes, transcription and translation occur in the same location whereas in eukaryotes it is separated
- Eukaryotes gene regulation occurs at different levels whereas in prokaryotes it occurs at transcriptional level

b. Explain the role of the leader region in E. coli's survival.

Marks	0	1	2	3	Average
%					

Any 2 of:
- Acts as a backup to repression/a checkpoint for basal trp production
- Allows for attenuation to occur
- Controls the production of tryptophan/transcription of structural genes
- Allows for formation of a terminator/attenuator **or** anti-terminator/anti-attenuator loop/allows the ribosome to pass or stop at the two trp codons
- Stops transcription/RNA polymerase detaches

and
- Conserve energy/prevent unnecessary protein production

Explain - function linked to the survival
No mark for restating survival

Exemplar student response: Leader region can control the expression of tryptophan through a process known as attenuation (forming antiterminator hairpin loops or terminator hairpin loops).
It helps with E.Coli's survival by conserving energy from unnecessary production of tryptophan.

c. Explain what scientists will need to consider when producing this synthetic molecule to ensure it appropriately inhibits tryptophan production.

Marks	0	1	2	3	Average
%					

Any two:
- Same amino acid sequence/primary structure
- Introns not included in gene
- Correct folding/creating of 2nd structures/formation of tertiary/3d/functional structure
- Conformational shape change needs to make repressor
- Bind to operator/be activated

And
- Repressor must block transcription/RNA polymerase/regulate gene expression

Proteins that aren't enzymes don't have active sites - they have binding sites

Question 4 (11 marks)
a. State if the Flavr Savr tomato produced by Calgene is a transgenic organism. Justify your response.

Marks	0	1	2	Average
%				

- Transgenic
- Gene inserted from a different species

b. Using the information in the flow chart, above, explain how CRISPR-Cas9 technology can be used to insert a gene to reduce the overripening of fruit.

Marks	0	1	2	3	4	Average
%						

Two of:
- sgRNA designed to be complementary DNA in Flavr Savr tomato
- sgRNA is combined with Cas9
- sgRNA guides Cas9
- Cas9; binds to PAM / cuts DNA
- Gene and CRISPR-Cas9 added to zygote of tomato plant

AND
- Gene inserted from different organism that produces a protein that inhibits/disrupts for ACC oxidase/ACC synthase/SAM synthase/inserting another gene to disrupt

AND (1 mark)
- Ethylene production stops / disrupted

No mark for restating stop overripening

Exemplar student response: Combine lab made sgRNA with complementary spacer sequence to the gene that produces ACC oxidase. Cas9 is obtained with appropriate PAM sequence to create a CRISPR-Cas9 complex. Inject this CRISPR-Cas9 complex into a specific cell, such as a zygote. Cas-9 will look for the gene that has a complementary PAM sequence and then make sure that the sequence aligns to that of the sgRNA. If it is complementary, the Cas-9 will cut the DNA strand. Then the gene will be inserted into the DNA resulting in no production of ACC oxidase, and if ACC oxidase cannot be formed then ethylene will also be unable to form, ultimately slowing down the over ripening of fruit.

c. With reference to the information, above, state which of the wells (A or B) has the modified gene. Justify your response.

Marks	0	1	2	Average
%				

- A
- Sample A DNA has a band (DNA) that hasn't travelled as far as B, suggesting that the correct gene has been inserted

d. Describe the function of the CRISPR sequence in bacteria.

Marks	0	1	2	3	Average
%					

Any three of the following:
- Cas1/Cas2 recognises PAM on the Viral DNA. Cas1/Cas2 cut the DNA upstream to the PAM region and make a protospacer which is 17-20 nucleotides in length, and will become a recognition sequence against the virus.
- Protospacer is embedded within the bacterial genome in the CRISPR region. Protospacer now considered a spacer.
- Spacer and repeated regions are transcribed into guideRNA,
- gRNA attaches to Cas9
- Cas9 will only read through viral DNA looking for PAM regions, if found, then will look for complementary sequence to the guideRNA.
- If complementary regions found, a cut is made a virus DNA destroyed = stop infection

Exemplar student response: Exposure: Cas ½ find viral DNA and inspect it's PAM sequence which is then cut generally 17-20 bases long creating a protospacer which then turns into a spacer in the CRISPR sequence.
Expression: After the CRISPR sequence is transcribed by RNA polymerase into gRNA, it is combined with the Cas9 enzyme.
Termination/Extermination: The Cas9 then searches for viral DNA which then inspects to see if a PAM sequence is present. If the viral DNA is complementary the Cas 9 cuts the viral DNA and induces a mutation.