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VCE Biology ¾
AOS 1 Revision [1.0]
SAC 3 Solutions

45 Marks. 5 Minutes Reading. 60 Minutes Writing.

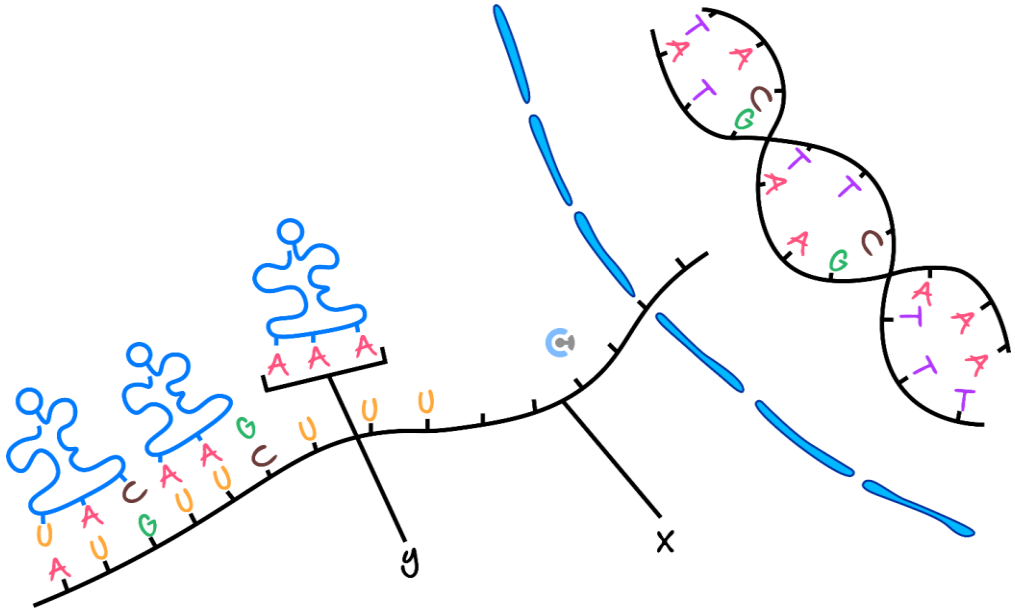
Section A: SAC Questions (45 Marks)

The Production of Human Insulin:

- In 1928, researchers discovered that insulin is a polypeptide, a finding that was further refined in 1952 when the precise amino acid sequence was determined. This hormone, critical for regulating metabolism, consists of two distinct protein chains: Chain A, which is made up of 21 amino acids, and Chain B, longer with 30 amino acids. Insulin's primary role is to facilitate anabolic processes, including the synthesis of proteins, which is essential for the growth and repair of skeletal muscle tissue. It plays a pivotal role in the body's management of nutrients, particularly by promoting the uptake of glucose into cells.

Question 1 (8 marks)

Insulin, like any other protein, is synthesised through transcription and translation of the genetic instructions that encode for it.



a. Name the complex responsible for the final step in the production of insulin polypeptides. (1 mark)

The ribosome is responsible for the final step in the production of insulin polypeptides.

b. What is label *Y*? (1 mark)

Anticodon

c. Outline the role of the complex named in **part a.** in the production of an insulin polypeptide. (3 marks)

The ribosome is responsible for synthesizing the insulin polypeptide through the process of translation:
The ribosome binds to the mRNA strand, reading the genetic code that specifies the sequence of amino acids in the insulin polypeptide.
tRNA molecules, carrying specific amino acids, bind to complementary mRNA codons in the ribosome, ensuring the correct amino acids are assembled.
The ribosome catalyzes the formation of peptide bonds between amino acids, elongating the polypeptide chain until a stop codon is reached, completing insulin synthesis.

The diagram above displays gene expression in eukaryotes.

d. What process is missing from this diagram? Name 2 events that occur during this process. (3 marks)

The missing process is RNA processing (post-transcriptional modification).
Two key events that occur during this process are:
Splicing – Introns (non-coding regions) are removed, and exons (coding regions) are joined together to form mature mRNA.
Addition of a 5′ cap and a poly-A tail – A modified guanine cap is added to the 5′ end for stability and ribosome binding, while a poly-A tail is added to the 3′ end to protect the mRNA from degradation.

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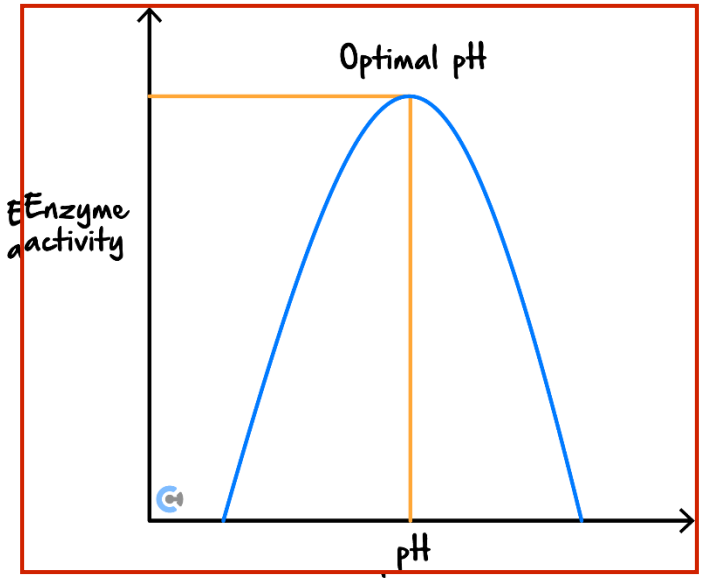
Insulin Production:

- The global surge in diabetes prevalence and advanced insulin administration methods, which often necessitate increased dosages, are anticipated to boost the demand for recombinant insulin. Existing production methods may struggle to meet this need affordably due to capacity constraints and elevated costs. To manufacture therapeutic recombinant insulin, a host organism is necessary–one capable of proper protein folding and post-translational modifications. Escherichia coli (E. coli) is commonly employed for producing recombinant human insulin for therapeutic use.

Question 2 (5 marks)

a. Sketch a graph showing enzyme activity against pH. (2 marks)



b. When bacterial growth is sufficient, scientists will often store bacteria at 4°C. Explain in terms of enzyme activity why scientists use this technique. (3 marks)

Scientists store bacteria at 4°C to slow bacterial metabolism and growth by reducing enzyme activity as:
Enzyme activity decreases at lower temperatures – Enzymes require kinetic energy to catalyze reactions, and at 4°C, molecular movement slows down, reducing enzyme-substrate interactions.
Slows bacterial growth and reproduction – Since bacterial enzymes are less active, essential metabolic processes slow down, preventing excessive growth and maintaining a stable bacterial population.
Preserves bacterial viability without killing them – Unlike high temperatures, which denature enzymes and destroy bacteria, low temperatures pause metabolic activity, allowing bacteria to be revived when needed.

Question 3 (10 marks)

a. Name three types of enzymes required to create recombinant DNA. Explain the role of each enzyme in creating a recombinant plasmid containing the human insulin gene. (6 marks)

Type of enzyme	Role in creating recombinant plasmid
Restriction Enzymes (Restriction Endonucleases)	These enzymes cut DNA at specific recognition sites, generating sticky or blunt ends. In creating a recombinant plasmid, a restriction enzyme cuts both the human insulin gene and the plasmid at complementary sites, allowing for the insertion of the insulin gene.
DNA Ligase	This enzyme joins DNA fragments together by forming phosphodiester bonds between the sugar and phosphate groups in the backbone. After the human insulin gene is inserted into the plasmid, DNA ligase seals the gaps, creating a stable recombinant plasmid.
DNA Polymerase	This enzyme synthesizes new DNA strands by adding complementary nucleotides. In recombinant DNA technology, DNA polymerase is used to amplify the insulin gene through PCR (Polymerase Chain Reaction) before insertion into the plasmid, ensuring there is enough DNA for successful cloning.

Plasmids must have a few key components in order to be considered as candidates for bacterial transformation.

b. Name ONE feature (excluding genes) that a plasmid must have to be used in the production of insulin, and describe their significance. (2 marks)

Origin of replication (ori)
The origin of replication (ori) is a DNA sequence that allows the plasmid to self-replicate independently within a bacterial cell. This ensures that multiple copies of the recombinant plasmid, carrying the human insulin gene, are produced, leading to efficient expression and large-scale insulin production.

c. Explain the feature of the genetic code that allows for insulin production to be performed using bacteria. (1 mark)

The genetic code is universal, meaning that the same codons code for the same amino acids in all organisms, allowing bacteria to produce human insulin.

d. Explain why there is an additional step of purification and modification when producing human insulin using E. coli. (1 mark)

E. coli lacks the ability to perform post-translational modifications, so insulin must be purified and correctly folded to become functional.

Definition

Insulin Ethics:

- Previously, insulin was extracted from the pancreas of pigs and cows until 1979.
  This emerged as a cost-effective mechanism that did not involve the killing of animals. However, there are still some reservations regarding the use of antibiotics, which may lead to antibiotic resistance.

Question 4 (9 marks)

a. Using the ethical concepts and principles you have learned this year, discuss the ethical implications of using recombinant plasmids to produce insulin. (4 marks)

The ethical implications of using recombinant plasmids to produce insulin can be evaluated through the following principles:
Beneficence (Doing Good) – Recombinant insulin provides a stable, reliable, and large-scale supply of insulin, benefiting millions of diabetics worldwide. It also reduces reliance on animal-derived insulin, which may cause allergic reactions.
Non-Maleficence (Do No Harm) – Although recombinant insulin is generally safe, there are concerns about the potential risks of genetically modified bacteria, such as antibiotic resistance or unintended mutations affecting safety.
Justice (Fair Distribution of Benefits and Burdens) – While recombinant insulin improves accessibility, its high cost may limit availability in low-income countries, raising concerns about equitable access to essential medicine.
Animal Welfare & Environmental Impact – Unlike traditional methods using animal pancreas, recombinant insulin reduces animal suffering. However, concerns about genetically modified bacteria escaping into the environment require strict containment measures.

b. Would E.coli be considered transgenic in this case? Explain. (2 marks)

Yes, E. coli would be considered transgenic because it has been genetically modified to contain the human insulin gene, which comes from a different species. (1 mark)
A transgenic organism is defined as one that has foreign DNA inserted into its genome, allowing it to express a gene from another species. In this case, E. coli produces human insulin due to the inserted gene. (1 mark)

c. Suggest a method by which insulin could be produced with plasmids without using antibiotics. (3 marks)

Auxotrophic Selection Method

1. The plasmid carries a gene that complements a metabolic deficiency in the host bacteria, such as the ability to synthesize an essential amino acid (e.g., leucine or tryptophan). (1 mark)
2. The bacteria used are auxotrophic mutants, meaning they cannot grow without supplementation of the missing nutrient unless they carry the plasmid. (1 mark)
3. By growing the bacteria in minimal media lacking the essential nutrient, only those containing the plasmid can survive and produce insulin, eliminating the need for antibiotics. (1 mark)

Definition

Insulin Ethics:

- Insulin, a hormone synthesised by pancreatic cells, plays a crucial role in the regulation of blood sugar. When one eats, blood sugar levels spike, prompting insulin release, which aids in glucose uptake by cells. Conversely, a drop in insulin prompts the liver to release glucose into the bloodstream. Inadequate insulin production or utilisation can precipitate diabetes mellitus.
  A healthy adult's pancreas typically harbours around 200 units of insulin, secreting 30 to 50 units daily. Insulin release is triggered by various physiological cues, with blood sugar levels being a primary regulator.

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Question 5 (6 marks)

a. The trp operon is one such example of gene regulation in E.coli.

Suggest 2 benefits of performing gene regulation. (2 marks)

Energy Conservation – Gene regulation prevents the unnecessary production of proteins, saving cellular energy and resources. (1 mark)
Environmental Adaptation – It allows bacteria to respond to environmental changes, such as the presence or absence of tryptophan, ensuring survival and efficient metabolism. (1 mark)

b. What is an operon? (1 mark)

An operon is a cluster of genes under the control of a single promoter, allowing coordinated regulation and expression of related genes in prokaryotes. (1 mark)

c. Explain how trp production in E.coli is regulated by repression of the trp operon. (3 marks)

When tryptophan levels are high, tryptophan binds to the trp repressor protein, activating it.
The activated repressor then binds to the operator region of the trp operon, blocking RNA polymerase from transcribing the genes needed for tryptophan synthesis.
This prevents unnecessary tryptophan production, conserving energy and resources. (3 marks)

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Question 6 (2 marks)

Outline the difference between a consequences-based and a duty/rule-based approach to resolving bioethical issues.

A consequences-based approach (utilitarianism) focuses on the outcome of an action, aiming to maximize overall benefit and minimize harm.
A duty/rule-based approach (deontology) emphasizes following moral rules or duties, regardless of the consequences, ensuring ethical principles are upheld. (2 marks)

Question 7 (1 mark)

Scientists are using blue-white screening to identify bacteria that have successfully taken up a recombinant plasmid containing the human insulin gene. In this experiment, the lacZ gene was intentionally interrupted by inserting the insulin gene within it. After transformation, bacterial colonies are grown on an agar plate containing X-gal.

Which of the following results would indicate that a bacterial colony has successfully taken up the recombinant plasmid containing the insulin gene?

A. A blue colony because the lacZ gene is still functional and able to break down X-gal.

B. A white colony because the inserted insulin gene has disrupted the lacZ gene, preventing β-galactosidase production.

C. No colony growth because the insulin gene prevents bacteria from dividing.

D. A fluorescent colony because insulin-producing bacteria glow under UV light.

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Question 8 (1 mark)

Which feature of a plasmid ensures that it is replicated and passed on to bacterial daughter cells after transformation?

A. The restriction enzyme recognition site, which ensures only transformed bacteria can grow.

B. The origin of replication (ORI), which allows the plasmid to be copied independently of the bacterial chromosome.

C. The selectable marker, which allows plasmids to be inherited through bacterial reproduction.

D. The promoter region, which initiates transcription and ensures plasmid replication.

Question 9 (1 mark)

During heat shock transformation, scientists treat bacterial cells with Ca^{2+} (calcium ions) before applying a brief heat pulse.

What is the main role of Ca^{2+} ions in this process?

A. Ca^{2+} strengthens the bacterial cell wall, preventing damage during heat shock.

B. Ca^{2+} activates heat shock proteins, which bind to plasmids and transport them into the bacterial cytoplasm.

C. Ca^{2+} neutralises the negative charge of the bacterial membrane and plasmid DNA, allowing DNA to enter the cell.

D. Ca^{2+} integrates into the plasmid DNA, making it more stable and easier to replicate.

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Question 10 (1 mark)

Scientists have inserted the human insulin gene into a plasmid and transformed E. coli cells. To confirm that the plasmid now contains the insulin gene, they use restriction enzymes to cut the plasmid and then run the DNA fragments through gel electrophoresis.

Which of the following results would confirm that the insulin gene was successfully inserted?

A. Two DNA bands: One corresponding to the plasmid backbone and one corresponding to the insulin gene.

B. A single DNA band matching the size of the original unmodified plasmid.

C. No DNA bands at all because recombinant plasmids do not migrate in a gel.

D. Multiple identical DNA bands across all lanes because all DNA fragments migrate the same distance in gel electrophoresis.

Question 11 (1 mark)

A biotechnology company is producing recombinant human insulin using E. coli. Initially, the bacteria are grown in large fermentation tanks, but the scientists notice that insulin production decreases significantly over time. They suspect that the plasmid containing the insulin gene is being lost from bacterial cells as they divide.

Which of the following would be the most effective strategy to ensure that bacterial cells retain the plasmid and continue producing insulin?

A. Growing the bacteria in a medium containing an antibiotic that selects for plasmid-containing cells.

B. Attaching the insulin gene to the bacterial chromosome instead of using a plasmid to ensure it is passed on during cell division.

C. Adding extra glucose to the medium to provide bacteria with more energy for plasmid replication.

D. Growing the bacteria in a medium containing X-gal that selects for plasmid-containing cells.

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