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VCE Biology ¾

AOS 1 Revision [1.0]

SAC 10 (JMSS) Solutions

33 Marks. 5 Minutes Reading. 45 Minutes Writing.

Section A: SAC Questions (33 Marks)

Question 1 (10 marks)

Use of DNA Technology in the Production of Human Insulin in Cow's Milk:

Recently, scientists genetically altered a brown bovine cow to produce human insulin in its milk. Insulin plays a crucial role in regulating blood sugar levels, and its function is to move glucose from the bloodstream into cells for energy. People with diabetes often require insulin, but it is expensive and not always accessible. Using DNA technology, researchers are exploring how to increase insulin production so that it can be more readily available to those who need it.

In order to express human insulin in bovine milk, researchers first constructed a recombinant plasmid, pREC, which contains the human insulin (hINS) gene placed downstream of the bovine β-casein 5 gene promoter **(Figure 1)**. Casein is a protein found specifically in mammalian milk. The plasmid pREC also contains several restriction enzyme recognition sites **(Figure 1)** and the blasticidinR and ampR genes **(Table 1)**.

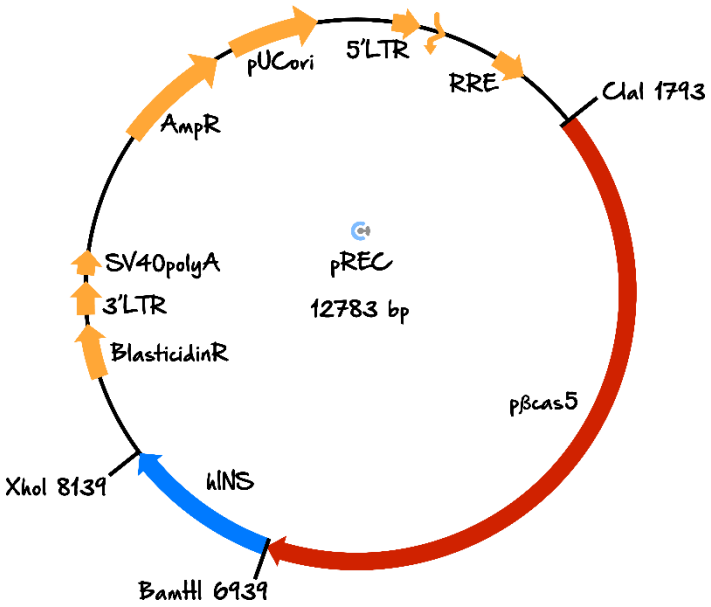

Figure 1: *Map of the recombinant plasmid,* pREC, *which is* 12,783 *base pairs (bp) in length and was introduced into a transgenic cow. Restriction sites for the endonucleases* ClaI, BamHI, *and* XhoI, *along with their positions on the map, are shown. The restriction sites have different recognition sites and produce sticky ends on cut DNA.*

DNA Region	Function
hINS	Human insulin gene (with 3 exons and 2 introns)
pβcas5	Bovine β-casein 5 promoter
BlasticidinR	Gene conferring resistance to blasticidin (antibiotic)
AmpR	Gene conferring resistance to ampicillin (antibiotic)

Table 1: *Functions of Selected DNA Regions Found in* pREC

To construct pREC, researchers performed the polymerase chain reaction (PCR) with the hINS gene and pβcas5 promoter region. They prepared two separate reaction tubes so as not to mix up the hINS gene and the pβcas5 promoter.

a. What is the purpose of PCR? (1 mark)

To amplify DNA samples

b. Describe how PCR works. (4 marks)

NAME OF STEP	TEMPERATURE (°C)	DESCRIPTION OF THE PURPOSE FOR THIS STEP
Denaturation	90°C-95°C	To break the hydrogen bonds between the double stranded DNA to form two single stranded DNA
Annealing	50°C-55°C	Primers to bind to the 3' end and acts as a starting point for elongation
Elongation	72°C	To copy or extend the DNA strand using taq polymerase (heat resistant DNA polymerase)

c. The two separate reaction tubes (for making multiple copies of hINS and pβcas5, respectively) contain essential ingredients (including chemicals and/or molecules).

i. Identify two common components that are found in BOTH tubes. (2 marks)

Buffer solution and taq polymerase

ii. Identify two unique components that are found in one tube and NOT found in the other tube. (2 marks)

- Primers are different (complementary to human insulin gene/casein promoter or gene of interest)
- DNA template is different (human OR insulin gene/cow OR casein promoter)
- HURDLE: Give 0.5 marks if insulin (casein) or human (cow) is NOT specified for each dot point (lose up to 1 mark)

d. Name the enzyme that joins hINS and pβcas5 gene regions together in **Figure (1)**. (1 mark)

(DNA) Ligase

Question 2 (7 marks)

To verify that pREC is correctly constructed, researchers digested pREC plasmid DNA with the restriction endonucleases ClaI, BamHI, and XhoI. The digested plasmid DNA was separated by gel electrophoresis, and three fragments ($X, Y,$ and Z) were observed **(Figure 2)**.

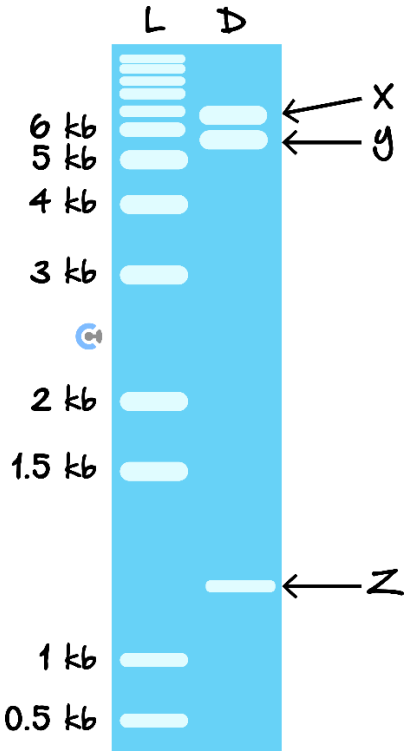

Figure 2: *Gel Electrophoresis of* pREC *plasmid DNA digested with* ClaI, BamHI, *and* XhoI (Lane *D*). Lane *L contains a DNA ladder with the indicated fragment sizes.*

Note: $1\ kb = 1000\ bp$.

a. Identify the DNA fragment (***X*****,*****Y*****, or*** ***Z***) that contains:

i. Human insulin (hINS) gene: (1 mark)

Z

ii. pβcas5 promoter: (1 mark)

Y

b. With reference to **Figure 2.**, explain how gel electrophoresis is used to separate DNA fragments and determine their sizes. (3 marks)

- ☑ DNA is **negatively charged** → move towards the positive/away from negative electrode (cause & effect)
- ☑ **Smaller DNA** fragments will move **further** than larger fragments or vice versa
- ☐ Use of **ladder/standard** to accurately determine size of fragments
  - ☐ HURDLE: To get full marks, students must provide an accurate estimate of at least one fragment's size e.g. X is bigger than 6kb, Y is between 5-6kb, Z is between 1-1.5kb.

c. After confirming that pREC was correctly constructed, researchers introduced pREC into bacterial cells. Describe how bacterial cells that were transformed with pREC were identified from those that did not take in the pREC plasmid. (2 marks)

- ☑ Cells are plated on agar/media containing one (**blasticidin/ampicillin**) or both antibiotics
- ☑ Cells that received pREC plasmid will be able to grow on antibiotic(s) or vice versa

Question 3 (5 marks)

Milk is produced by the mammary glands in mammals. To produce human insulin in cows' milk, scientists genetically modified bovine mammary cells so that they contained a copy of pREC. Nuclear DNA from these cells was transferred into embryos. The embryos were implanted into surrogate cows. A transgenic calf was born **(Figure 3)**, and upon maturity, scientists detected human insulin in its milk.

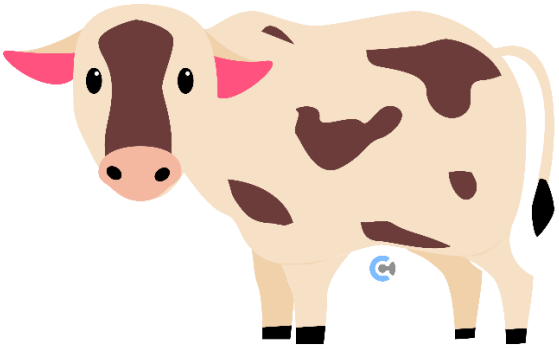

Figure 3. Transgenic bovine calf genetically modified to produce human insulin.

a. Describe the features of the genetic code that allow us to take the insulin gene in humans and put it into cows to make the same protein. (2 marks)

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_____

- ☑ Genetic code is **universal**.
- ☐ The **triplets/codons**/sequence of **3 bases** for each amino acid are the same (in humans and cows)

_____

b. In an individual who does not have diabetes, the human insulin gene is regulated by its own promoter and is only expressed in pancreatic cells. With reference to the structure of pREC **(Figure 1)** and its components **(Table 1)**, explain the key components of pREC that enable the transgenic cow's mammary cells to produce milk containing insulin. (3 marks)

_____

- ☐ Identify **casein** promoter
- ☐ (Casein) promoter allows insulin to be made by cells/in a location that is not normally possible. *Insufficient to state promoter required for milk to contain insulin (already in Q prompt).*
- ☑ Human insulin gene is placed **downstream**/3' of casein promoter (or vice versa) OR is **regulated** by/under the **control** of the casein promoter (B)
- ☐ HURDLE: For full marks, students must indicate the positions of the casein promoter and human insulin gene relative to each other.

_____

Question 4 (7 marks)

Transcription of the human insulin gene in transgenic bovine cells begins in the nucleus (refer to **Figure (1)** and **Table (1)**). Insulin protein synthesis is initiated by translating insulin mRNA in the cytoplasm to produce the polypeptide called preproinsulin.

a. Before the export of insulin mRNA into the cytoplasm, describe the modifications that occur in the nucleus. (3 marks)

- ☐ Addition of **5'** methyl G cap/5' **cap**
- ☐ Addition of **3' poly-A tail**
- ☑ **Removal of introns** (2) AND **exons** remain (3)
  - ☐ HURDLE: To get full marks, students must mention the number of exons (3) and introns (2) in the human insulin gene, as specified in Table 1. Only 0.5 mark if the numbers of exons and introns are not specified.

b. Identify the organelle where translation occurs. (1 mark)

Q4b. Identify the organelle where translation occurs.

- ☑ Ribosome or rough endoplasmic reticulum (ER) *incorrect to imply smooth endoplasmic reticulum hence "rough"*

c. Preproinsulin undergoes post-translational modifications and is converted into proinsulin. Proinsulin is eventually secreted out of the cell.

i. Identify the organelle in which preproinsulin is converted to proinsulin. (1 mark)

rough endoplasmic reticulum (ER)

ii. Identify the structure in which proinsulin is transported within the cell. (1 mark)

Vesicles

iii. Identify the process by which proinsulin is secreted out of the cell. (1 mark)

Exocytosis

Space for Personal Notes

Question 5 (4 marks)

The scientific community has raised several ethical issues related to the use of transgenic cows to produce human insulin. For each comment raised below, indicate ONE ethical concept that best describes the type of implication/issue. Each concept may only be used ONCE.

Comments and Questions Raised by Individuals	Ethical Concept
With regard to mass-producing insulin in milk, there is a need for specialised, expensive high-health-status facilities for the productio of insulin.	Justice - affordability of treatment costs
Genetic engineering has so much potential for significant advancements in how we understand, diagnose, and treat diseas This study highlights the promise this field has in applications f improving human health.	Beneficence - improving human health
Producing insulin in bacteria (e.g., E. coli) and yeast is more huma and does not require the use of embryos or transgenic animal	Non-maleficence - harming/destroying cow embryos
We need to stop exploiting animals. Leave the cows and their bodies alone!	Respect - care for the cattle and their bodies

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