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Email: hello@contoureducation.com.au

VCE Biology ¾
AOS 1 Revision [1.0]
SAC 5 Solutions

42 Marks. 5 Minute Reading. 60 Minutes Writing.

Section A: SAC Questions (42 Marks)

CRISPR Could Create Hypoallergenic Cats

The results of a recent study found that genetically engineering cats could be a solution to eliminating cat allergies.

- **The fur-midable cause of cat allergies: Fel d 1**

Over several decades, scientists have identified eight cat allergens, the molecules that trigger allergies - none, however, are more notorious than Fel d 1. This nasty little protein accounts for up to 96% of human allergic reactions to cats. Cats secrete it through almost every opening that allows secretions: the mouth, skin and even their eyes. Furthermore, all cats produce Fel d 1 regardless of breed, age, sex or housing (indoors v/s outdoors). In other words, there is no such thing as an allergen-free cat.

Genome engineering with CRISPR gene editing is a practical approach for tackling cat allergies as it can selectively inactivate specific genes; however, there is a catch - Fel d 1's biological function is unknown and it is rarely a good idea to delete genes when you don't know what they do.

- **Com-paw-ing domestic to exotic cats**

To determine if Fel d 1 is essential, the researchers at InBio compared the two genes that code for this protein allergen, CH1 and CH2, in domesticated cats to distantly related exotic cats. When genes are important, they are usually conserved across evolutionary time. That is, if Fel d 1 is essential to a cat's health, then the genes that encode it should be similar in domesticated cats and exotic cats.

For their domestic cat sample, the researchers used discarded tissue samples (feline testes, ovaries and uteri) from the desexing procedures of 50 domestic cats. They compared the CH1 and CH2 sequences to those of exotic cat genomes available in the *National Centre for Biotechnology Information* database. The exotic cat species included a subset spanning several lineages of the family *Felidae*, ranging from members of the same genus as domestic cats to species that diverged from domestic cats around 11 million years ago. The scientists found that CH1 and CH2 sequences varied widely across the groups, suggesting that Fel d 1 is not essential and can, therefore, be eliminated without causing any health issues.

Next, the scientists developed CRISPR methods to inactivate both CH1 and CH2. These methods were up to 55% effective at mutating the genes in cells grown in the lab. Additionally, the scientists found no evidence that the method unintentionally edited other genes, which could have nasty consequences for a cat's health.

Engineering hypoallergenic cats

"Taken together, our data indicate that Fel d 1 is both a rational and viable candidate for deletion, which may profoundly benefit cat allergy sufferers by removing the major allergen at the source," concluded the authors. Perhaps, one day, the elusive hypoallergenic cat will become a reality.

Evolutionary Biology and Gene Editing of Cat Allergen, Fel d 1

Introduction

The domestic cat (*Felis catus*) is the most common source of mammalian allergen, with cat allergies affecting up to 15% of people and producing symptoms *that range from rhinoconjunctivitis (a runny and blocked nose) to severe asthma. Recent data from the US *National Health and Nutrition Examination Survey* attributed more than 500,000 asthma attacks per year and ~350,000 emergency care visits to patients who were sensitised and exposed to cat allergens. Despite the prevalence and potential severity of cat allergic disease, current treatments merely address the allergic symptoms (e.g. antihistamines) or have demonstrated inconsistent results.

The major cat allergen, Fel d 1, is a protein and natural levels of the allergen vary significantly between cats (> 100-fold) and even within the same cat. While the biological function of Fel d 1 is unknown, studies suggest that the protein may play a role in chemical communication, epithelium defence (layers of cells that line the surfaces of the body) or immune regulation. To date, there have been no documented knockouts of Fel d 1 in cats.

Genome engineering presents a unique opportunity for the deletion of Fel d 1 from cat cells and tissues and may provide the critical step in establishing the definitive function of the allergen. CRISPR, in particular, offers considerable improvements in target specificity, editing efficiency and precision compared to prior technologies. Additionally, CRISPR has demonstrated promise in therapeutic applications (e.g. sickle cell disease) and offers profound clinical potential. Given the advantages of CRISPR gene editing and a specific genomic target in Fel d 1, CRISPR is a practical approach for tackling cat allergic diseases, which may greatly improve the lives of cat-allergic individuals.

Discussion and Conclusions

Our CRISPR knockouts in feline test cells demonstrate that Fel d 1 is a viable target for gene deletion, which is expected to improve the health of cat-allergic patients substantially by effectively removing the allergen at the source. Several recent approaches to cat allergy aim to neutralise Fel d 1 and reduce cat allergen exposure.

In the future, the CRISPR knockouts will be replicated in Fel d 1-expressing primary feline cells and, eventually, in living cats. Future studies will aim to develop a means for deleting the Fel d 1 genes in adult cats, effectively rendering the cats hypoallergenic, while further analyses of proteins from other mammalian species may provide additional insight regarding the precise biological function of Fel d 1.

To date, this is the most comprehensive bioinformatics analysis of any allergen and the first study to investigate the evolutionary origins of an allergen. Our data illustrate a practical application of CRISPR technology in allergy research or veterinary medicine and underscore the unique intersection between evolutionary biology, genome engineering and therapeutic development.

Question 1 (7 marks)

CRISPR gene editing was used to target the CH1 and CH2 genes, which encode the Fel d 1 protein, a major cat allergen. Scientists investigated whether deleting these genes would affect feline health.

a. What is the function of DNA in an organism? (1 mark)

DNA stores genetic information and provides instructions for the synthesis of proteins that control cellular functions and organismal traits.

b. The CRISPR process involves modifying the CH1 and CH2 genes in the cat genome. What is a genome, and how is it related to an organism's phenotype? (2 marks)

A genome is the complete set of genetic material in an organism, including all of its genes. Genes within the genome encode proteins that determine an organism's traits, which collectively contribute to its phenotype.

c. Translation is the process that converts mRNA into a functional protein. Describe the key steps of translation. (3 marks)

1. **Initiation** – The ribosome assembles around the mRNA, and the first tRNA binds to the start codon (AUG), bringing the corresponding amino acid.
2. **Elongation** – tRNA molecules continue to bring amino acids that match the mRNA codons, and the ribosome links them together to form a polypeptide chain.
3. **Termination** – When a stop codon is reached, translation ends, and the newly synthesised protein is released.

d. Scientists compared the amino acid sequences of Fel d 1 in domestic and exotic cats. Why do proteins with different amino acid sequences still perform similar functions? (1 mark)

Proteins with different amino acid sequences can still have similar functions if their overall shape and active sites remain unchanged. This is because protein function is determined by its three-dimensional structure rather than just the sequence alone.

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Question 2 (8 marks)

The article "*Evolutionary Biology and Gene Editing of Cat Allergen, Fel d* 1" states:

"Genome engineering presents a unique opportunity for the deletion of Fel d 1 *from cat cells and tissues and may provide the critical step in establishing the definitive function of the allergen."*

a. Which ethical principle is most relevant when considering the potential benefits of deleting the Fel d 1 gene? Justify your response. (2 marks)

- **Beneficence:** This principle refers to maximising benefits while minimising harm.
- Removing Fel d 1 could provide significant benefits by reducing allergic reactions in humans, improving the quality of life for cat allergy sufferers. If the gene is non-essential for feline health, its deletion could be considered ethically justified.

b. Apart from this, explain another ethical principle that should be considered when deciding whether to genetically modify cats. (2 marks)

- **Justice:** This principle ensures fair treatment and equitable distribution of benefits and risks.
- Scientists must consider whether hypoallergenic cats will be accessible to all individuals or if they will be limited to those who can afford expensive gene-edited pets. Additionally, the welfare of the animals must be balanced against human benefits.

c. CRISPR-Cas9 is widely used as a gene-editing tool but originally evolved as part of a bacterial immune system. Identify two differences between CRISPR's natural role in bacteria and its use in genetic engineering. (2 marks)

1. **In bacteria, CRISPR targets and cuts viral DNA as a defense mechanism**, while in genetic engineering, it is used to **precisely modify an organism's own DNA**.
2. **Bacteria acquire CRISPR sequences naturally from viral infections**, whereas scientists design specific guide RNAs to **direct Cas**9 **to a chosen gene for targeted modifications.**

d. Scientists used reverse transcriptase to create DNA from the mRNA of Fel d 1 before gene editing. Explain why they used mRNA instead of DNA. (2 marks)

- **mRNA has already undergone splicing**, meaning non-coding introns have been removed, making it easier to analyse and manipulate.
- **It ensures that only the functional, protein-coding regions of the gene are studied**, avoiding unnecessary sequences that could interfere with CRISPR targeting.

Question 3 (6 marks)

The Fel d 1 protein, a major cat allergen, is composed of multiple chains and encoded by the CH1 and CH2 genes. Scientists used CRISPR to study its genetic structure and function.

a. The Fel d 1 protein consists of multiple polypeptide chains. What level of protein structure does this represent? (1 mark)

Quaternary Structure: This level of protein structure occurs when multiple polypeptide chains interact and form a functional protein.

b. Each chain of the Fel d 1 protein is made up of 70 amino acids. How many nucleotides would be required to code for a single chain in mature mRNA? Provide evidence for your response. (2 marks)

- Each amino acid is encoded by **three nucleotides (one codon)**.
- 70 amino acids × 3 nucleotides per amino acid = **210 nucleotides**.
- Additionally, a **stop codon (3 nucleotides)** is needed, bringing the total to 213 **nucleotides**.

c. The scientists used discarded tissue samples to compare the genomes of domestic cats with those of exotic cats. Whilst this may be considered unethical as the cats are unable to consent, their research suggests that the Fel d 1 gene is not essential and as such can be eliminated.

What ethical approach are the scientists employing? Justify your response with reference to all three approaches. (3 marks)

- **Consequences-based approach**: This approach prioritises maximising benefits and minimising harm. The scientists argue that removing Fel d 1 benefits many people by reducing allergies while causing no harm to cats if the gene is non-essential.
- **Duty/rule-based approach**: This approach focuses on ethical rules regardless of consequences. From this perspective, genetic modification of animals might be deemed unethical if altering a species for human benefit is considered inherently wrong.
- **Virtue-based approach**: This approach considers individual morals and ethical values. Some may argue that **respect for animal well-being** should come before human benefits, suggesting genetic modification should be avoided unless it directly benefits the animal.

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Question 4 (9 marks)

During the pGlo experiment, some bacteria were transformed to allow them to glow under UV light. The plasmid acted as a vector to insert the gene for pGlo into the bacterium.

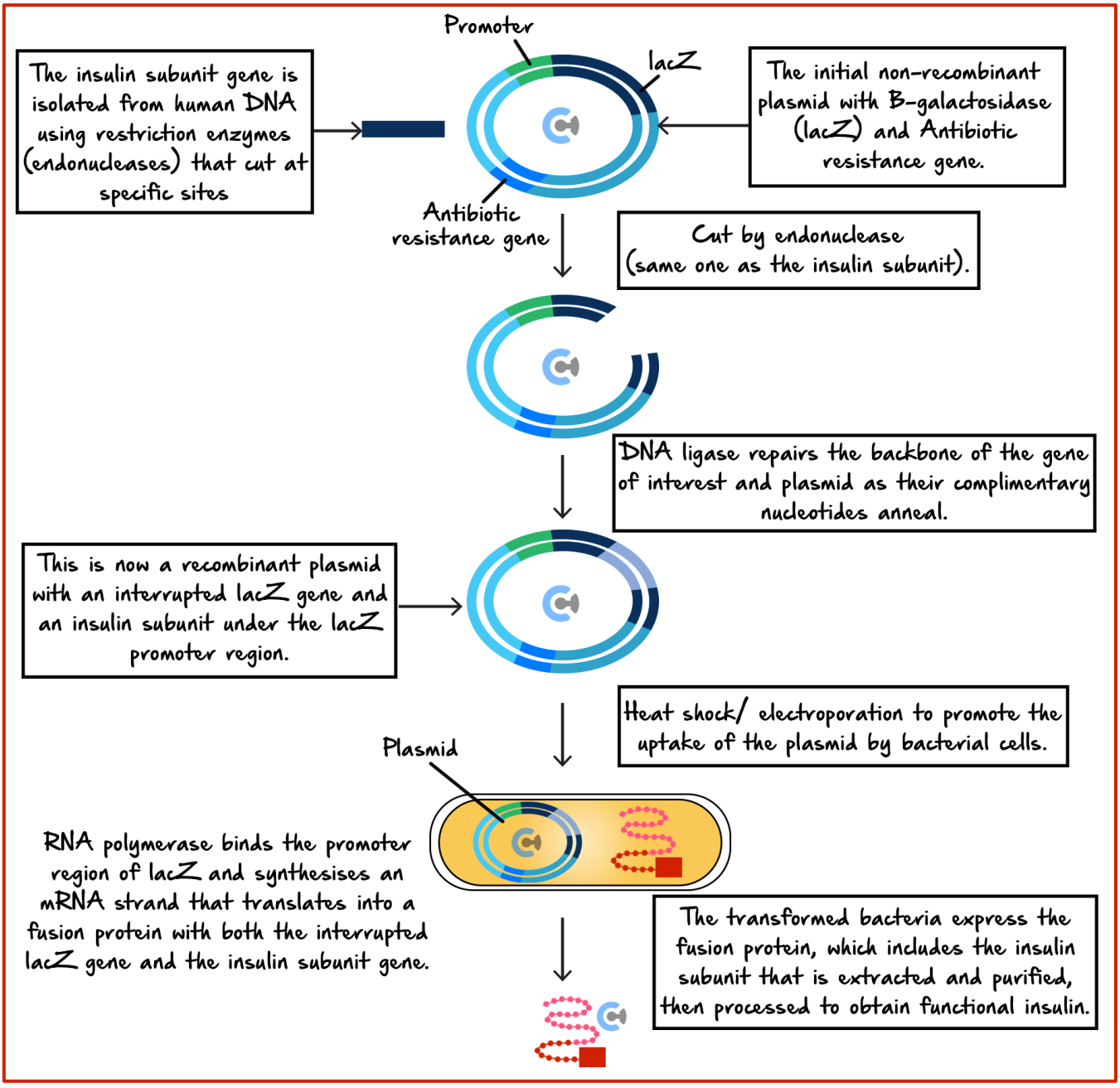
a. When the plasmid was cut, sticky ends were created. What is the advantage of using sticky ends instead of blunt ends in recombinant DNA technology? (1 mark)

Sticky ends have **overhanging sequences**, which ensure **specific base pairing**, leading to more accurate and efficient insertion of the desired gene.

b. In bacterial transformation experiments, scientists often use antibiotic-resistance genes as selectable markers. Explain what a selectable marker is and why antibiotic-resistance genes are commonly used. (4 marks)

- A **selectable marker** is a gene introduced into an organism that allows scientists to identify and isolate cells that have successfully taken up foreign DNA.
- **Antibiotic resistance genes** are commonly used because only bacteria that contain the recombinant plasmid will survive when grown on antibiotic-containing media.
- This allows scientists to **easily distinguish transformed bacteria** from those that did not take up the plasmid, ensuring that only the desired genetic modifications are studied.

c. Recombinant plasmids are also used to produce insulin, with a number of steps required to transform the resultant bacterium. The diagram below shows the synthesis of one of these chains. Label the diagram using the boxes to provide a brief overview of this process. (3 marks)



d. Would a bacterium containing a recombinant plasmid for insulin production be considered genetically modified (GM) or transgenic? Justify your response. (1 mark)

- The bacterium is **transgenic** because it contains a gene from a different species (human insulin gene).
- It is also **genetically modified (GM)** because its DNA has been altered, but the term **transgenic** specifically applies when a gene from another species is introduced.

Question 5 (7 marks)

The Fel d 1 protein is produced in cat cells and secreted through various bodily secretions, triggering allergic reactions in humans. Scientists are investigating whether **CRISPR** could be used to **permanently switch off the genes** responsible for producing Fel d 1.

However, an alternative approach, **CRISPR interference (CRISPRi)**, allows for gene suppression **without permanently altering DNA**. Unlike traditional CRISPR, which uses the Cas9 enzyme to cut DNA, CRISPRi uses a **deactivated Cas9 (dCas9)** that binds to gene regions and **prevents transcription** without making irreversible changes.

In bacteria, **gene expression is naturally regulated** by operon systems, such as the **trp operon**, which controls tryptophan production.

a. Explain how the trp operon naturally regulates gene expression and compare how CRISPRi could be used to suppress Fel d 1 in a way that mimics this system. (5 marks)

- The **trp operon** is a **repressible system** that is normally active but **turns off when tryptophan is present**.
- When tryptophan levels are high, a **repressor protein binds to the operator**, preventing RNA polymerase from transcribing the genes needed to make tryptophan.
- Similarly, **CRISPRi can be used to silence the Fel d 1 genes (C1H1 and CH2) by directing dCas9 to their promoter regions**, blocking RNA polymerase from transcribing them.
- If the CRISPRi system is **removed or inactivated**, **Fel d 1 expression could resume**, just like the trp operon **restarts transcription when tryptophan levels drop**.
- The **key difference** is that the **trp operon is a natural feedback system**, while **CRISPRi is artificially designed by scientists** to **control gene expression on demand**.

b. Why might scientists prefer using CRISPRi instead of permanently deleting the Fel d 1 genes with traditional CRISPR? (2 marks)

- **CRISPRi allows reversible gene suppression**, meaning that if future research shows Fel d 1 has an essential function, its expression can be restored.
- Permanent deletion using CRISPR **introduces irreversible mutations**, which could **unintentionally disrupt feline biology** and affect health.

Question 6 (5 marks)

With any DNA analysis technique, it is essential to have a sufficient amount of DNA to work with before conducting further tests.

a. State the full name of the process used to amplify DNA samples before analysis. (1 mark)

Polymerase Chain Reaction (PCR)

A scientist is analysing a DNA fragment that is **15 kilobases (*kb*) long**. They treat the sample with a **restriction enzyme** that makes **two cuts**: one at **4 *kb* from one end** and another at **10 *kb* from the same end**.

b. How many DNA fragments will be produced after enzyme digestion? (1 mark)

Three fragments (One fragment of 4 kb, one of 6 kb, and one of 5 kb).

A **gel electrophoresis setup** is used to separate these DNA fragments by size.

c.

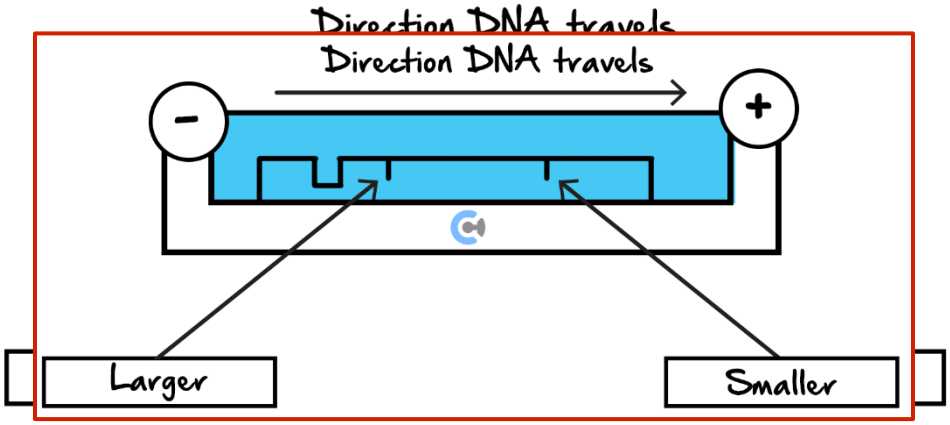
i. Label the positive and negative electrodes in a gel electrophoresis chamber. (1 mark)

- **Negative electrode (−)** is at the **starting point of the DNA samples** (since DNA is negatively charged).
- **Positive electrode (+)** is at the **opposite end of the gel** where DNA fragments migrate toward.

ii. Label the DNA fragments as larger or smaller relative to each other. (1 mark)

- **The 6 *kb* fragment is the largest, followed by the 5 *kb*, and the 4 *kb* is the smallest.**
- **Smaller fragments travel further in the gel, while larger fragments move more slowly.**

A gel is set up to enable the fragments to be analysed. The gel can be seen below.



d. How does the viscosity of the gel affect the migration speed of DNA fragments? (1 mark)

- **A higher viscosity (thicker gel) slows down DNA migration**, making bands more distinct.
- **A lower viscosity (thinner gel) allows DNA to move faster**, but resolution between bands may decrease.

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