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VCE Biology ¾
AOS 1 Revision [1.0]
SAC 5

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)

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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)

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

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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)

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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)

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

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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)

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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)

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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)

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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)

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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)

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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)

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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)

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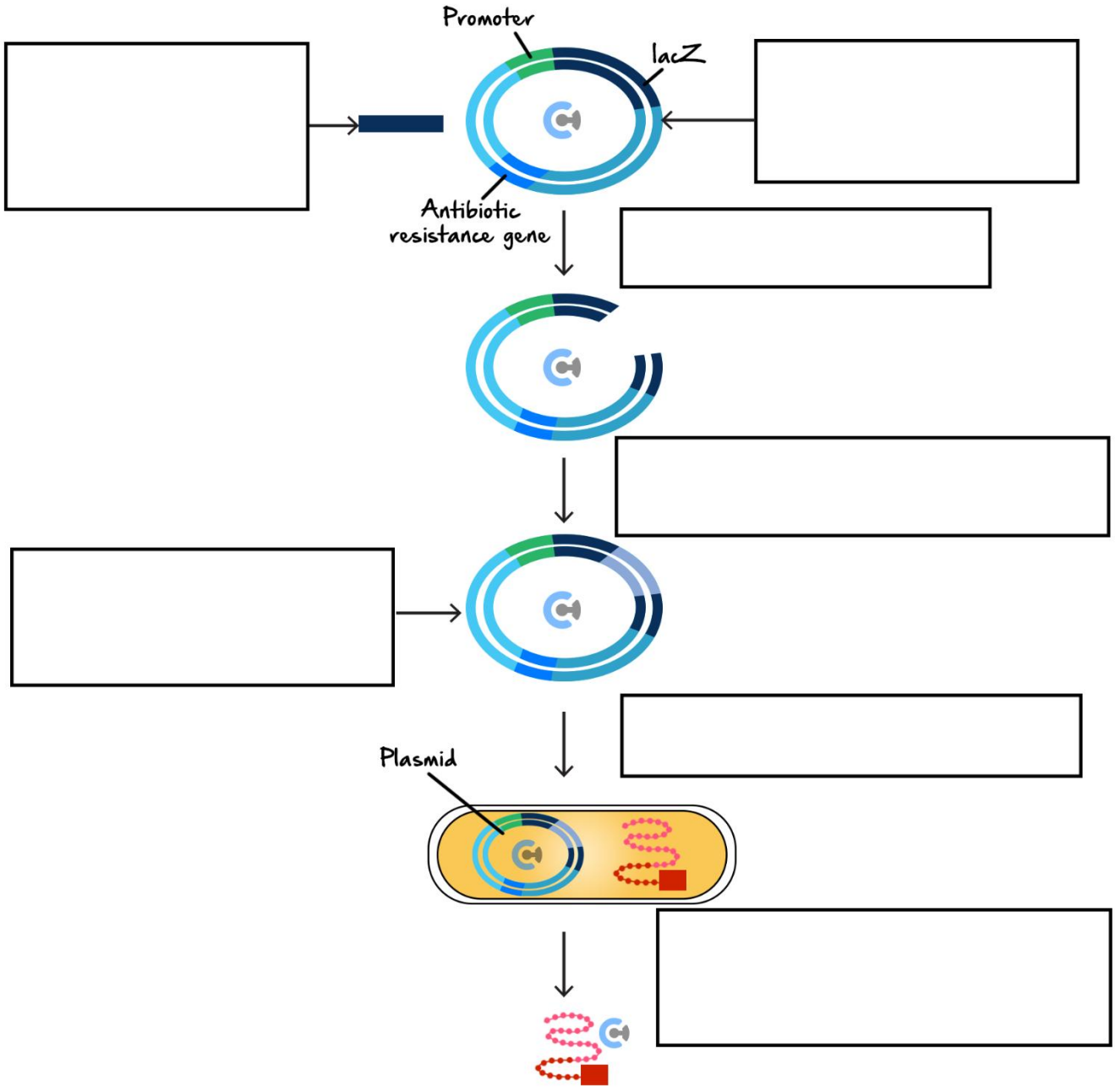
c. Recombinant plasmids are also used to produce insulin, with a number of steps required to transform the resultant bacterium. The diagram below shows the synthesis of one of these chains. Label the diagram using the boxes to provide a brief overview of this process. (3 marks)



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

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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)

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

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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)

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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)

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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)

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

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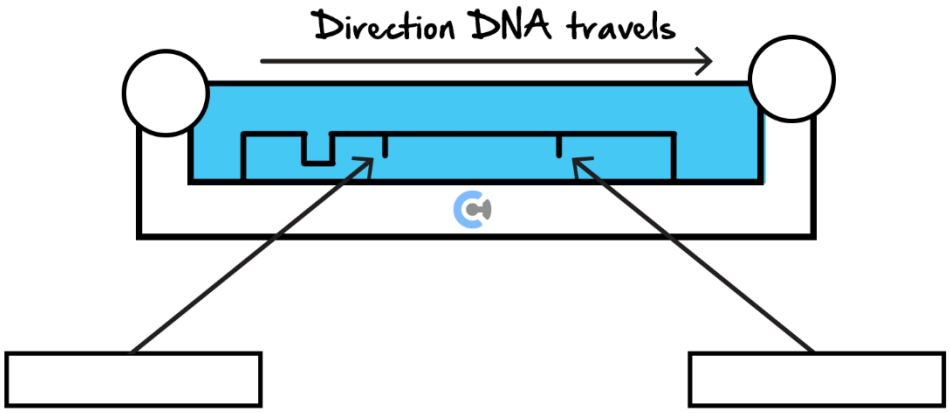
A gel is set up to enable the fragments to be analysed. The gel can be seen below.



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

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