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VCE Biology ¾

CRISPR-Cas9 & Bioethics [1.6]

Homework Solutions

Homework Outline:

Compulsory Questions	Pg 1 – Pg 21

Section A: Compulsory Questions (41 Marks)

Sub-Section [1.6.1]: Describe the Function and the Process of CRISPR-Cas9 as an Adaptive Defense Against Viruses in Bacteria

Question 1

a. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats):

A region in the bacterial genome containing short, repetitive DNA sequences separated by unique "spacer" sequences derived from viral DNA. This system stores a record of past viral infections, forming the basis for adaptive immunity in bacteria.

b. Cas Proteins:

A group of enzymes that work with CRISPR sequences to recognise and cut foreign DNA. Cas9 is the most well-known protein, which acts as “molecular scissors” to cleave specific DNA sequences.

c. Spacer DNA:

Unique DNA sequences inserted into the CRISPR array that are derived from viral genomes. These sequences act as a memory of past infections and guide the system to target matching viral DNA during future infections.

d. Guide RNA (gRNA):

A small RNA molecule transcribed from the CRISPR array that guides the Cas9 protein to its complementary DNA sequence in the invading virus, ensuring precision in targeting.

e. Adaptive Immunity:

A defence mechanism an organism uniquely targets different pathogens, and is also able recognise and respond more effectively to subsequent infections by the same pathogen.

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Question 2 (1 mark)

What is the primary function of CRISPR-Cas9 in bacteria?

A. To synthesise proteins for metabolic processes.

B. To act as an adaptive defense against viral infections.

C. To enable bacterial conjugation.

D. To promote genetic variation through mutations.

Question 3 (1 mark)

How does the CRISPR-Cas9 system recognises and targets viral DNA?

A. It binds randomly to any DNA in the cell.

B. It synthesises new RNA molecules that directly destroy viral DNA.

C. It uses RNA sequences transcribed from CRISPR arrays to guide the Cas9 protein to complementary viral DNA.

D. It relies on physical contact with viral proteins to detect and target viral DNA.

Question 4 (5 marks)

a. A student states that Cas9 is involved in incorporating a proto spacer into the CRISPR array in bacteria. Explain where the student has gone wrong, and correctly describe the function of the Cas9 enzyme. (2 marks)

- Cas1 and Cas2 enzymes cut out the proto spacer.
- Cas9 is involved in cleaving the viral DNA upon re-exposure to a virus.

b. Alternatively, describe how the CRISPR-Cas9 functions as a defense system in bacteria. (3 marks)

- Upon infection, viral DNA is injected into the bacterium by a bacteriophage and Cas1 and Cas2 enzymes cleave a section of viral DNA (near to a PAM sequence but not including). This viral DNA is incorporated into the bacterium's CRISPR array.
- The CRISPR array is transcribed into gRNA which combines with a Cas9 enzyme to form the CRISPR Cas9 complex.
- Upon re-infection, the gRNA that is complementary to the viral DNA guides the Cas9 enzyme, that cleaves the virus' DNA until eventually inducing a mutation that inactivates the virus.

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Sub-Section [1.6.2]: Explain the Process of Using CRISPR-Cas9 as a Gene Editing Tool, Including Silencing, Knock-Ins & Knock-Outs

Question 5

a. Gene editing:

The deliberate alteration of a specific DNA sequence in the genome. This can involve adding, deleting, or altering genetic material.

b. Knock in:

A process where CRISPR-Cas9 is used to introduce a new gene or DNA sequence into a specific location in the genome.

c. Knock out:

A gene-editing technique that disables or removes a gene's function. CRISPR-Cas9 achieves this by creating double-strand breaks in DNA, followed by repair processes that introduce errors or deletions.

d. Mutation:

A change in the DNA sequence. In CRISPR-Cas9 applications, mutations are often introduced intentionally to disrupt or modify gene function.

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Question 6 (1 mark)

What is a knock-out in the context of CRISPR-Cas9 gene editing?

A. Inserting a new gene into a specific location in the genome.

B. Silencing gene expression by targeting RNA.

C. Disrupting a gene's function by introducing a mutation or deletion.

D. Replacing a defective gene with a functional one.

Question 7 (1 mark)

How does CRISPR-Cas9 facilitate gene knock-ins?

A. By silencing genes through blocking transcription.

B. By cutting DNA and inserting new genetic material during repair.

C. By using guide RNA to degrade messenger RNA before translation.

D. By introducing random mutations to alter gene expression.

Question 8 (2 marks)

Researchers used CRISPR-Cas9 to modify the GROW1 gene, which regulates plant height. They designed a guide RNA targeting the gene's coding region but did not provide a donor DNA template for repair. After the experiment, the plants showed no detectable GROW1 protein, and their growth was significantly stunted compared to controls.

Which CRISPR-Cas9 technique was likely used in this experiment? Justify.

- Knock out.
- The GROW1 is no longer detected and it has not been replaced with a different allele.

Sub-Section [1.6.3]: Describe & Compare the Function of the PAM Sequence in Bacteria & Gene Editing Applications of CRISPR-Cas9 Technology

Question 9

Protospacer Adjacent Motif (PAM):

A short DNA sequence adjacent to the target site in the viral genome, required for Cas9 recognition and cutting. Without the PAM sequence, Cas9 will not cleave the DNA, ensuring specificity.

Question 10 (1 mark)

What is the primary function of the PAM sequence in the natural bacterial CRISPR-Cas system?

A. To prevent Cas9 from cutting bacterial DNA by recognising self-DNA.

B. To allow Cas9 to bind any DNA sequence, regardless of its origin.

C. To direct the synthesis of guide RNA (gRNA) for Cas9.

D. To recruit repair enzymes after DNA cutting.

Question 11 (1 mark)

How does the PAM sequence influence the application of CRISPR-Cas9 in gene editing?

A. It ensures Cas9 can bind any DNA sequence, enhancing versatility.

B. It limits the sequences Cas9 can target by requiring a PAM near the target site.

C. It eliminates the need for guide RNA (gRNA) in targeting specific DNA.

D. It repairs DNA after Cas9 introduces a double-stranded break.

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Question 12 (3 marks)

a. Compare and contrast the role of the PAM sequence in both bacteria and in gene editing. (2 marks)

The PAM (Protospacer Adjacent Motif) sequence is essential in the bacterial CRISPR-Cas system to distinguish foreign DNA from self-DNA. Cas9 requires the PAM sequence adjacent to the target DNA for binding and cleavage, ensuring that bacterial DNA, which lacks a PAM near stored spacer sequences, is not targeted. In gene-editing applications, the PAM sequence determines where Cas9 can bind and cut DNA, limiting target sites to those with a PAM nearby. This ensures specificity but also constrains the flexibility of targetable DNA sequences.

b. Other than preventing self-harm to the bacteria, what role does the PAM sequence play naturally? (1 mark)

Efficiency - Cas9 searches for PAM sequences.

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Sub-Section [1.6.4]: Describe the Function & Compare the Guide RNA (gRNA) & Single Guide RNA (sgRNA)

Question 13

a. sgRNA:

A synthetic RNA molecule combining crRNA and tracrRNA into a single strand for simplified use in CRISPR applications.

b. CRISPR RNA (crRNA):

- A short RNA molecule that contains a sequence complementary to the target DNA.
- Function: Guides the Cas9 protein to the specific DNA sequence to be cleaved.

c. Trans-activating CRISPR RNA (tracrRNA):

- A structural RNA molecule that binds to the crRNA and helps form a complex with the Cas9 protein.
- Function: Stabilises the interaction between crRNA and Cas9, enabling the system to function effectively.

Question 14 (1 mark)

What is the primary difference between guide RNA (gRNA) and single guide RNA (sgRNA)?

A. gRNA is made purely from RNA nucleotides, while sgRNA is composed of both RNA and DNA components.

B. gRNA is naturally occurring in bacteria, while sgRNA is artificially engineered.

C. gRNA is used only in gene editing, while sgRNA is used only in bacteria.

D. gRNA has higher specificity than sgRNA.

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Question 15 (1 mark)

How may sgRNA be designed to minimise the chance of offsite cuts?

A. By designing a shorter spacer region in the sgRNA to increase flexibility in target binding.

B. By avoiding the inclusion of a PAM sequence in the sgRNA.

C. By designing a longer spacer region in the sgRNA to maximise specificity in target binding.

D. By eliminating the scaffold region of the sgRNA to reduce binding efficiency.

Question 16 (2 marks)

Provide one structural and one functional difference between gRNA and sgRNA.

- crRNA and tracrRNA are fused in sgRNA whilst they exist as separate molecules in gRNA.
- gRNA is utilised in bacteria's natural defense whereas sgRNA is utilised in gene editing.

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Sub-Section [1.6.5]: Apply Bioethical Principles to the Use of CRISPR-Cas9 Technology

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Sub-Section [1.6.6]: Define & Describe the Bioethical Concepts of Integrity, Respect, Beneficence, Non-Maleficence & Justice as Elaborated in the VCAA Study Design

Question 17

a. Beneficence:

- The principle of doing good and acting in ways that benefit individuals and society.
- Application in CRISPR-Cas9:
  - Using CRISPR to develop treatments for genetic disorders or enhance human health.
  - Ensuring that research and applications maximise positive outcomes, such as curing diseases or improving agricultural yields to address food security.

- The principle of avoiding harm or minimising the potential for harm.
- Application in CRISPR-Cas9:
  - Preventing unintended consequences, such as off-target edits that could introduce harmful mutations.
  - Ensuring the safety of gene-editing applications before clinical use to avoid risks to patients or ecosystems.

c. Respect:

- Acknowledging the autonomy, dignity, and rights of individuals and other living organisms.
- Application in CRISPR-Cas9:
  - Obtaining informed consent from individuals involved in gene-editing research.
  - Respecting the rights of future generations by avoiding irreversible or harmful genetic modifications.
  - Considering ethical treatment of animals in CRISPR research.

d. Justice:

- Fair and equitable distribution of benefits, risks, and resources.
- Application in CRISPR-Cas9:
  - Ensuring that the benefits of gene-editing technologies are accessible to all, not just privileged groups.
  - Addressing potential disparities in access to CRISPR therapies, especially for marginalised populations or in low-income countries.

e.

- Upholding honesty, transparency, and accountability in research and application.
- Application in CRISPR-Cas9:
  - Conducting gene-editing research ethically and reporting results accurately.
  - Avoiding misuse of CRISPR for unethical purposes, such as creating "designer babies" or unauthorised modifications.
  - Adhering to international guidelines and regulations for gene-editing technology.

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Question 18 (1 mark)

A pharmaceutical company develops a CRISPR-Cas9 therapy to cure a life-threatening genetic disorder. While the therapy shows promising results, it is priced so high that only wealthier patients can afford it. Advocacy groups raise concerns about equitable access to the therapy, arguing that it should be made available to all affected individuals, regardless of their financial situation. Which ethical principle is most relevant in addressing these concerns?

A. Non-maleficence - ensuring the therapy does not cause harm to patients.

B. Justice - ensuring fair and equitable access to the therapy.

C. Beneficence - maximising positive outcomes for patients who can afford it.

D. Integrity - maintaining honesty in reporting the benefits of the therapy.

Question 19 (1 mark)

A CRISPR-Cas9 clinical trial aims to modify the germline DNA of embryos to prevent a heritable genetic disease. Although the researchers have obtained proper regulatory approval and documented short-term safety, there is significant uncertainty about how these genetic modifications might affect future generations. Critics argue that the potential risks outweigh the benefits. Which ethical principle is primarily being questioned in this scenario?

A. Beneficence - ensuring the research provides significant benefits.

B. Respect - acknowledging the autonomy of the individuals involved.

C. Non-maleficence - avoiding harm, particularly to future generations.

D. Justice - ensuring equal access to the trial for affected families.

Question 20 (1 mark)

A team of scientists uses CRISPR-Cas9 to study a genetic mutation by editing the genomes of laboratory animals. They ensure that the research is carefully designed to minimise suffering, obtain the maximum amount of useful data, and contribute to life-saving therapies. However, animal rights activists challenge the experiment, claiming it violates ethical standards. The researchers defend their work by emphasising that all animals are treated humanely, and their study is aimed at improving human health. Which ethical principle best supports the researchers' position?

A. Respect - ensuring the humane treatment of animals in the experiment.

B. Justice - ensuring the benefits of the study are shared across populations.

C. Integrity - conducting the research honestly and transparently.

D. Beneficence - ensuring the research maximises potential benefits for society.

Question 21 (4 marks)

Scientists are exploring the use of CRISPR-Cas9 technology to modify the genes of livestock to enhance meat production efficiency. For example, CRISPR could be used to increase muscle growth or disease resistance in cattle. However, concerns have been raised about the long-term welfare of the animals and the potential ecological consequences of releasing genetically modified organisms into the environment.

Discuss an ethical concern that could be raised about the use of CRISPR-Cas9 technology in livestock. Propose a feasible solution to this ethical concern. State the ethical concept or approach that has been addressed in your discussion.

- Unintended off cuts utilising CRISPR-Cas9 may result in animals being harmed/inheriting deleterious genes.
- This is related to the principle of non-maleficence.
- Non-maleficence involves minimising any foreseeable harms, and in this case minimising any suffering experienced by the livestock.
- A feasible solution here is to perform extensive trials with CRISPR technology perhaps on other organisms. In addition, companies should slowly start to implement using this technology before using it on widespread farms.

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Sub-Section [1.6.7]: Define & Describe the Three Ethical Approaches as Elaborated in the VCAA Study Design

Question 22

- This approach evaluates the morality of an action based on its outcomes or consequences. An action is considered ethical if it leads to the greatest good for the greatest number of people (also known as utilitarianism).
- Example: In the context of CRISPR-Cas9, using gene editing to eradicate a hereditary disease might be considered ethical if the positive outcomes (e.g., improved quality of life) outweigh the risks or harms.

b. Virtues-Based Approach:

- This approach assesses ethical decisions based on the character and intentions of the individual or group taking the action, rather than the outcomes. It focuses on cultivating moral virtues such as honesty, compassion, and courage.
- Example: Scientists using CRISPR responsibly and transparently, driven by integrity and compassion for patients, reflects a virtues-based approach.

c.

- Definition: Also known as deontology, this approach focuses on following moral rules, duties, or obligations regardless of the consequences. An action is ethical if it adheres to these principles.
- Example: Opposing the use of CRISPR-Cas9 on embryos might align with a duty-based approach if the action is viewed as violating the moral rule to respect the dignity and autonomy of future generations, even if the outcomes could be beneficial.

Question 23 (1 mark)

A biotechnology company proposes using CRISPR-Cas9 to engineer crops that are more resistant to drought and pests. While there are concerns about the long-term environmental impact of genetically modified organisms, the company justifies its decision by highlighting the potential to address food shortages in drought-prone regions and reduce reliance on harmful pesticides.

What ethical approach is primarily guiding the company's decision?

A. Duty-based approach - prioritising adherence to ethical rules about environmental protection.

B. Consequences-based approach - focusing on maximising benefits like food security.

C. Virtues-based approach - emphasising compassion for affected communities.

D. Respect-based approach - ensuring the autonomy of farmers to choose improved crops.

Question 24 (1 mark)

A research team refuses to edit human embryos with CRISPR-Cas9 despite significant funding and pressure from investors. They argue that doing so would prioritise financial gain over ethical responsibility. Instead, they focus on projects that reflect their values of integrity and respect for human life, even though these projects may have less immediate impact.

What ethical approach is primarily guiding the research team's decision?

A. Consequences-based approach - weighing financial risks and potential benefits.

B. Justice-based approach - ensuring fairness in research funding allocation.

C. Duty-based approach - adhering to rules against germline editing.

D. Virtues-based approach - prioritising ethical character and moral values.

Question 25 (1 mark)

An international organisation bans the use of CRISPR-Cas9 to create genetically modified organisms in agriculture, citing an obligation to follow global agreements that prohibit certain forms of genetic modification until further studies confirm safety. The decision is made without considering specific potential benefits or risks in individual cases.

What ethical approach is primarily reflected in this decision?

A. Virtues-based approach - demonstrating a commitment to caution and responsibility.

B. Consequences-based approach - ensuring the technology benefits the majority.

C. Duty-based approach - adhering to established ethical rules and guidelines.

D. Justice-based approach - promoting fairness in decision-making.

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Question 26 (4 marks)

A research team is considering the use of CRISPR-Cas9 to modify the genes of crops to improve drought resistance. Some team members argue that the potential to reduce global hunger justifies the risks of unintended ecological consequences, while others prioritise strict adherence to existing regulations that limit genetic modification.

Identify and explain two ethical approaches being debated by the research team. State how each approach influences the decision-making process.

The two ethical approaches being debated are the consequences-based approach and the duty-based approach.
Solutions

1. Consequences-Based Approach:
   This approach focuses on the potential benefits and harms of the action. Team members advocating for CRISPR-Cas9 use emphasise the positive outcomes, such as reducing global hunger by creating drought-resistant crops. They argue that the benefits to society, including improved food security, outweigh the risks of unintended ecological consequences.
2. Duty-Based Approach:
   This approach emphasises adherence to moral rules and existing regulations. Team members prioritising the regulations believe that it is unethical to proceed with genetic modifications if it violates agreed-upon guidelines, regardless of the potential benefits. They argue that following these rules is essential to ensure responsible and fair use of genetic technology.

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Sub-Section [1.6.8]: Describe Briefly How to Genetically Modify Organisms to Increase Crop Productivity & Disease Resistance, Using CRISPR-Cas9

Question 27

a. Crop Productivity:

- The ability of crops to produce a higher yield (quantity of produce per unit area) or grow more efficiently under given conditions.
- Crop productivity refers to genetic modifications that enhance plant traits such as growth rate, fruit size, nutrient content, or tolerance to environmental stresses like drought or salinity.

b. Disease Resistance:

- The ability of crops to withstand or minimise damage caused by pathogens such as bacteria, viruses, fungi, or pests.
- Disease resistance involves genetic modifications using CRISPR-Cas9 to enhance the plant's defense mechanisms or eliminate susceptibility to diseases.

Question 28 (1 mark)

A scientist wants to increase drought tolerance in wheat using CRISPR-Cas9. What is the first step in the genetic modification process?

A. Introducing the CRISPR-Cas9 components into the plant cells using a gene gun.

B. Identifying the specific gene responsible for drought tolerance in wheat.

C. Regenerating modified plants using tissue culture techniques.

D. Designing a gRNA complementary to the plant's entire genome.

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Question 29 (1 mark)

Which of the following best describes how CRISPR-Cas9 increases disease resistance in crops?

A. By eliminating the need for traditional pesticides through improved water uptake.

B. By creating a random mutation in the plant's genome to remove harmful traits.

C. By editing susceptibility genes or introducing new resistance genes into the plant genome.

D. By using Cas9 to repair DNA damage caused by environmental stresses.

Question 30

You are a plant geneticist working to help tomato farmers combat devastating losses caused by fungal infections from Fusarium oxysporum. Research shows that the **S (Susceptibility) gene SITOM1** plays a critical role in facilitating fungal infections in tomatoes. By editing this gene using CRISPR-Cas9, you aim to reduce the plant's susceptibility to fungal pathogens.

Describe how you would use CRISPR-Cas9 to modify the SITOM1 gene to enhance fungal resistance in tomatoes.

Steps in the CRISPR-Cas9 Process:

1. Research has identified SITOM1 as a key susceptibility gene in tomatoes that supports fungal infection.
2. A gRNA is designed to specifically bind to the DNA sequence of the SITOM1 gene. This ensures precise targeting by the CRISPR-Cas9 complex.
3. The Cas9 protein and gRNA are introduced into tomato plant cells using methods like Agrobacterium-mediated transformation or a gene gun.
4. The Cas9 protein, guided by the gRNA, introduces a double-strand break at the SITOM1 gene. The plant's natural DNA repair mechanisms inactivate the gene by introducing small errors (non-homologous end joining).
5. The modified tomato cells are grown into full plants using tissue culture techniques. These plants are tested to confirm the inactivation of SITOM1 and their enhanced resistance to fungal infections.

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Sub-Section [1.6.9]: Compare Transgenic, Cisgenic & Genetically Modified Organisms

Question 31

a. Transgenic Organism:

- An organism that has been genetically modified by inserting a gene (or genes) from a different species.

b. Cisgenic Organism:

- An organism that has been genetically modified using a gene from the same species or a closely related one that could naturally interbreed with it.

c. Genetically Modified Organism (GMO):

- A broad term referring to any organism whose genetic material has been altered using genetic engineering techniques.

Question 32 (1 mark)

Which of the following best describes a **cisgenic organism**?

A. An organism modified with a gene from an unrelated species to introduce a new trait.

B. An organism modified with a gene from the same species or a closely related one.

C. An organism modified without introducing any new genetic material.

D. An organism modified with synthetic DNA sequences designed in the lab.

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Question 33 (1 mark)

A farmer grows a new type of wheat that has been genetically modified to resist fungal infections by introducing a gene from a bacterium. This organism is an example of:

A. A genetically modified organism (GMO) only.

B. A cisgenic organism.

C. A transgenic organism.

D. A naturally bred organism.

Question 34 (1 mark)

Which of the following statements is true about genetically modified organisms (GMOs)?

A. All GMOs involve inserting genes from unrelated species.

B. GMOs only include cisgenic organisms.

C. GMOs encompass all organisms whose genetic material has been altered, including modifications to endogenous genes.

D. GMOs must always involve crossing natural species barriers.

Question 35 (2 marks)

A group of scientists develops a new variety of apples that are resistant to fungal infections. To achieve this, they introduce a gene from a wild apple species into a commercial apple variety that is sexually compatible. The gene enhances the plant's ability to produce antifungal compounds.

Based on the genetic modification described, determine whether this apple variety is transgenic or cisgenic.

- The apple variety is cisgenic.
- This is because the introduced gene comes from a wild apple species, which is sexually compatible with the commercial apple variety, and not from an unrelated species.

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