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VCE Biology ¾

CRISPR-Cas9 & Bioethics [1.6]

Test Solutions

45 Marks. 1 Minute Reading. 40 Minutes Writing.

Results:

Test Questions	_____ / 45

Section A: Test Questions (45 Marks)

Question 1 (5 marks)

Tick whether the following statements are **true** or **false**.

Statement	True	False
a. CRISPR-Cas9 originates from a bacterial immune system designed to defend against viruses.	✓	
b. The guide RNA (gRNA) directs Cas9 to its target by complementary base pairing with the viral DNA.	✓	
c. The protospacer adjacent motif (PAM) is not necessary for Cas9 to bind and cut DNA.		✓
d. CRISPR-Cas9 cannot be used to make precise edits to an organism's genome.		✓
e. Non-homologous end joining (NHEJ) is a repair mechanism that introduces errors, often silencing genes.	✓	
f. Ethical concerns about CRISPR include the inability of embryos to consent to genome edits.	✓	
g. Virtue-based ethics focus on the outcomes of actions rather than the character of the individual.		✓
h. GMOs are always created using CRISPR-Cas9 technology.		✓
i. Beneficence involves minimising risks while maximising benefits in scientific practices.	✓	
j. Cross-pollination between GM and non-GM crops cannot occur under any circumstances.		✓

Sub-Section: Multiple Choice Questions

Question 2 (1 mark)

Bacteria use CRISPR-Cas9 as an adaptive immune system against viruses. Imagine a scenario where a bacterium is exposed to a new virus. Which sequence of events best describes how CRISPR-Cas9 protects the bacterium?

A. The bacterial cell immediately destroys all viral DNA using Cas9.

B. The bacterial cell stores fragments of the viral DNA as spacers, transcribes them into RNA and guides Cas9 to cut matching viral DNA during subsequent infections.

C. The bacterial cell incorporates viral DNA into its genome to make itself immune to future infections.

D. The bacterial cell produces multiple Cas9 enzymes to eliminate the virus directly upon first contact.

Question 3 (1 mark)

Certain plants, like bananas and potatoes, possess genes that produce proteins conferring resistance to specific plant diseases. In a bid to enhance crop resilience, researchers have isolated these disease-resistance genes and inserted them into different plant species that are typically vulnerable to those diseases.

How are the modified plants, which now contain genes for disease resistance obtained from other species, referred to?

A. Hybrid

B. Transgenic

C. Polyploid

D. Recombinant

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Question 4 (1 mark)

Which of the following best describes how CRISPR-Cas9 is able to allow for the insertion of a desired gene?

A. CRISPR-Cas9 binds to the target DNA, cuts it, and allows random mutations to create the desired gene.

B. CRISPR-Cas9 uses the guide RNA to locate the target DNA, and cut it, and the cell's repair mechanisms incorporate a provided template containing the desired gene.

C. CRISPR-Cas9 directly inserts the desired gene into the genome without cutting the DNA.

D. CRISPR-Cas9 uses PAM sequences to synthesise the desired gene and incorporate it into the genome.

Question 5 (1 mark)

CRISPR-Cas9 uses a guide RNA to target specific DNA sequences. How does the guide RNA ensure specificity, and what would happen if the RNA sequence were only partially complementary to the target DNA?

A. The guide RNA aligns perfectly with any DNA sequence, allowing flexibility in targeting.

B. The guide RNA must be fully complementary to the target DNA; partial matches reduce cutting efficiency and increase off-target risks.

C. The guide RNA adapts to new DNA sequences over time, improving targeting.

D. The guide RNA binds to PAM sequences regardless of the target sequence, ensuring cuts are made.

Question 6 (1 mark)

If a bacterial cell fails to incorporate viral DNA spacers into the CRISPR locus, which part of the adaptive immune response is compromised?

A. The ability to transcribe spacers into crRNA.

B. The recognition of future viral infections.

C. The activation of Cas9 to cut viral DNA.

D. The detection of PAM sequences in the bacterial genome.

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Question 7 (1 mark)

Imagine CRISPR-Cas9 is applied in agriculture to enhance drought resistance in crops. Which of the following outcomes demonstrates a biological implication of this technology?

A. Farmers save money on irrigation systems.

B. Public concern arises about the safety of genetically modified crops.

C. The edited crops are more resistant but may reduce genetic diversity in wild populations.

D. Agricultural companies charge higher prices for drought-resistant seeds.

Question 8 (1 mark)

A student argues that CRISPR-Cas9 should be used in all cases where it can improve human health. Which ethical principle could be used to challenge this statement?

A. Beneficence, because it supports maximising good outcomes.

B. Justice, because the benefits may not be equally accessible to everyone.

C. Non-maleficence, because no technology is free of risks.

D. Integrity, because scientific transparency is required.

Question 9 (1 mark)

In bacteria, why does the PAM sequence play a crucial role in distinguishing self from non-self DNA?

A. It prevents the bacteria from cutting its own genome by ensuring Cas9 only binds to foreign DNA.

B. It allows the bacteria to transcribe its own spacers into crRNA without interference.

C. It identifies viral DNA sequences that cannot be stored in the CRISPR locus.

D. It enhances the efficiency of RNA transcription in bacterial cells.

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Question 10 (1 mark)

A group of scientists proposes using CRISPR-Cas9 to enhance intelligence in humans. What social and ethical concerns does this raise, and how might justice be implicated?

A. Enhancing intelligence is purely a biological matter, so social implications are irrelevant.

B. Justice concerns arise because such technology may only be available to wealthy individuals, increasing inequality.

C. Social concerns focus on whether intelligence is heritable, which is unrelated to justice.

D. Ethical concerns would be minimal as long as experiments are transparent.

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Sub-Section: Short Answer Questions

Question 11 (12 marks)

CRISPR-Cas9 is an exciting new gene editing tool that has allowed for more specificity and control when editing the genetic material of organisms, both in the lab and also in live experiments.

In prokaryotes, where it was first isolated, it has been shown to have a significant immune role in the cell.

a. What does CRISPR do in prokaryotes? (1 mark)

Neutralises invading VIRAL DNA.

b. Explain how CRISPR-Cas9 works in prokaryotes to achieve its aim. (3 marks)

- A virus inserts its DNA into a bacterium, and a spacer is cut out and incorporated into the CRISPR locus.
- This is then transcribed to form crRNA which is then combined with tracrRNA to form guideRNA.
- This will form a CRISPR-Cas9 complex with Cas9 enzyme and float around the cell until it encounters complementary viral DNA.
- Viral DNA is cleaved and inactivated.

c. Describe the importance of having a PAM sequence for prokaryotes in the CRISPR-Cas9 system. (1 mark)

It prevents the bacterial chromosome from getting cut itself by Cas9.

Recently, in 2012, it was determined that CRISPR-Cas9 could be adapted from prokaryotes to provide an important tool for gene editing.

d. Explain what aspect of CRISPR allows it to be specific. (2 marks)

- The guide RNA can be synthesised in a lab to match a specific sequence.
- This sequence binding via complementary base pairing initiates this process.
- Presence of the PAM before cutting also ensures specificity.

CRISPR has been put forward as a potential treatment option for babies for genetic diseases such as cystic fibrosis, where a mutation to a single gene can cause lifelong disease.

e. Explain how CRISPR-Cas9 can be applied to embryos with the potential for cystic fibrosis identified early. (3 marks)

- Identify a target sequence for a cut (in this case the gene with the error for cystic fibrosis) and develop a synthetic guide RNA (sgRNA) complementary to it.
- Combine this with the Cas9 enzyme, altered with a PAM to suit the target.
- Inject this into a target cell (the embryo), and then the sgRNA will bind to the target DNA, and then signal the cut.
- Cell repair mechanisms will try and repair causing errors, which will silence the gene, theoretically preventing cystic fibrosis.

f. Compare the importance and use of the PAM in prokaryotes and gene editing. (2 marks)

PAM in prokaryotes = Prevents the chromosome from getting cut itself whilst improving efficiency of the cut (Cas9 doesn't have to search the ENTIRE sample for a sequence).

PAM in experiments = Can be altered to suit any specific target sequence, depending on the experiment, other than that doesn't really play much of a role except for the efficiency aspect as described above.

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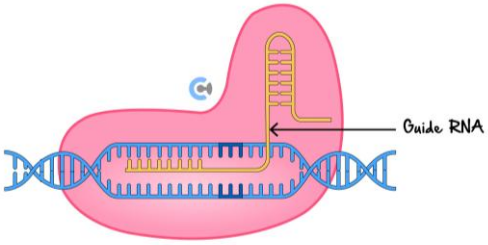
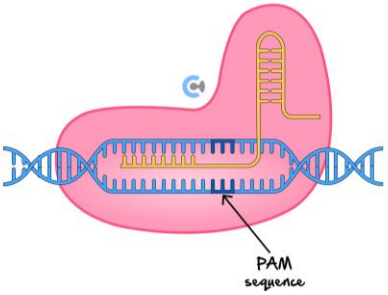
Question 12 (8 marks)

CRISPR-Cas9 is currently being studied to investigate potential treatments for leukemia patients. Leukemia is a type of cancer that affects the development and production of blood cells. Zebrafish have been used to model leukemia in order to investigate possible treatments for the disease. Scientists target and cut a specific gene area in Zebrafish that codes for a granulocyte colony-stimulating factor receptor protein (G-CSFR). This protein stimulates white blood cell production and is shortened in some leukemia patients. Through the creation of specific guide RNA and the use of CRISPR-Cas9, scientists have been able to induce the Zebrafish to develop the mutated version of the G-CSFR protein that is identical to the mutation in some leukemia patients. The mechanism the scientists used is referred to as gene knockout.

(1 mark) for correct labelling of guide RNA.



(1 mark) for correct labelling of guide PAM sequence.



a.

i. In the figure above, label the guide RNA scientists used to target the G-CSFR gene. (1 mark)

ii. In the figure above, label the PAM sequence. (1 mark)

b. Describe the function of guide RNA in targeting the G-CSFR gene. (2 marks)

The guide RNA enables the CRISPR-Cas9 complex to recognise the complementary target sequence of the G-CSFR gene (1 mark) and cleave at the specific site of the DNA (1 mark).

c. Outline how the process of 'gene knock out' works in the process of inducing leukemia in the Zebrafish. (3 marks)

Gene knock out is the process of using CRISPR-Cas9 to cut the DNA at the specific G-CSFR gene region and allowing the cell to repair the DNA (1 mark).
The cell will attempt to repair the DNA by randomly inserting nucleotides into the DNA (1 mark).
The insertion of random nucleotides will alter the base sequence of the gene and therefore, the amino acid sequence resulting in the G-CSFR exon that causes leukemia (1 mark).

d. Explain the role gel electrophoresis might play in the scientific process of inducing the G-CSFR mutation using CRISPR-Cas9 that causes leukemia in Zebrafish. (1 mark)

Gel electrophoresis is used to identify the DNA that has been mutated and shortened by CRISPR-Cas9 (1 mark).

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Question 13 (11 marks)

Unbeknownst to many, Angad manages Contour by day and is also a mad scientist by night. Trying to generate the next generation of Contour tutors, he decides to try some CRISPR-Cas9 experiments to create superhuman Contour tutors who might aid his goals of dominating the VCE tutoring market, involving the use of human embryos.

Before he can do this, however, he has to run through countless trials so that he can get regulatory approval.

a. What are the independent and dependent variables of his experiment? (2 marks)

Independent Variable – The edits made to the embryos.
Dependent Variable – Tutoring ability.
***Multiple answers possible cos it is a vague sort of scenario – ask yourself, can you define either of these terms?

b. Describe the relevance of the ethical principle of respect that Angad must consider when running his experiment. (3 marks)

The ethical principle of respect involves consideration to the extent to which people or things have an intrinsic or instrumental value, making sure that individual's rights to autonomy, welfare, liberty and culture are all respected.
In this experiment, Angad has to consider the respect for autonomy, and the capacity of living things to consent – to ensure that those that are getting edited are giving consent (whether this is possible or not given that they'll be embryos). This involves their "parents" as well-being fully informed about the risks and the dangers of the procedure.
Further, the respect of the value of human life is important as well – any editing mechanisms that he may apply have to be safe for the editing of embryos otherwise they might be wasted.

He didn't get favourable results so he decided to fake them, in order to try and convince the dreaded TGA that his program deserved approval.

c. Apply the principle of integrity and use a virtues-based approach to discuss the ethics of his decision. (4 marks)

Integrity – The commitment to searching for knowledge, and the honest reporting of all information, especially in regards to experiments, whether favourable or unfavourable, to permit the increase of understanding or public knowledge.

- This is not integrity being shown, as Angad is faking information of his results and is not contributing to public understanding.
- As he is trying to mislead the TGA, this lacks integrity as any approval based on false information will not actually result in public knowledge increasing, rather, he is doing this for his own financial benefit (his intentions are not good).

Virtues based approach involves the consideration to the actions that a good principle individual would take, referring to the moral character of the person involved – in this case, Angad.

- It is acceptable to say this action would be unethical for a good person to undertake, as it involves deliberately misleading and lying about results, both of which are values which are not considered to be held by those with high moral character.
- Further, judging the moral character of Angad himself, from the information presented in this scenario, his intentions for faking his result seem unethical, as he is primarily concerned about the financial incentive involved.

Angad eventually did not receive approval from the TGA and he decided to pursue his experiments unapproved in his lab.

d. Why is this unethical? (2 marks)

- There are a number of approaches that describe why this could be considered unethical. Show Aaliyan your answer for final marking or if there is any confusion.
- Unintended consequences of unapproved experiments – they may have not been safely tested for use and they had been rejected for a reason.
  - This affects any number of ethical principles, think about that.
- Setting a precedent for rejecting the authority of the TGA – may cause further issues such as other unapproved products being misleading, or impacting people's safety.

Disclaimer: This is not how this works, please do not try to be Angad.

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