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VCE Biology ¾

CRISPR Cas9 & Bioethics [0.7]

Workshop Solutions

Section A: Recap

Active Recall: What is the purpose of CRISPR-Cas9 in bacteria?

Active Recall: What is the purpose of CRISPR-Cas9 in gene editing?

Active Recall: Name and define the 5 "concepts" of bioethics in the study design.

Active Recall: Name and define the 3 "approaches" to bioethics in the study design.

Section B: Multiple Choice Questions (19 Marks)

Question 1 (1 mark)

CRISPR-Cas9 differs from traditional restriction enzymes, such as EcoRII:

A. It creates both sticky and blunt ends.

B. It does not have a set recognition site.

C. The recognition site is palindromic.

D. It can cut any type of nucleic acid.

CRISPR-Cas9 can cut at any sequence of DNA, whereas restriction enzymes can only cut at a specific palindrome sequence.

Question 2 (1 mark)

What is the role of CRISPR-Cas9 in prokaryotes?

A. It repairs damaged DNA within the cell.

B. It acts as an adaptive immune defence by targeting viral DNA.

C. It enhances the efficiency of protein synthesis in bacteria.

D. It integrates plasmid DNA into the bacterial genome.

Question 3 (1 mark)

Which of the following best describes how CRISPR-Cas9 is used to create a "gene knockout"?

A. A gene is silenced by introducing a mutation into its promoter region.

B. A gene is completely removed from the genome by Cas9 cutting it at multiple locations.

C. Cas9 cuts the DNA at a specific site, leading to errors during repair, which inactivate the gene.

D. Cas9 binds to the PAM sequence and blocks transcription of the gene.

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Question 4 (1 mark)

Why is the PAM sequence essential in CRISPR-Cas9 gene editing?

A. It stabilises the sgRNA-Cas9 complex.

B. It serves as a recognition site for the Cas9 protein to bind and cut the DNA.

C. It is part of the sgRNA sequence that guides Cas9 to the target site.

D. It allows the insertion of foreign DNA into the genome.

Question 5 (1 mark)

Which of the following represents an ethical concern in the use of CRISPR-Cas9 technology?

A. The potential to accidentally modify off-target genes.

B. The lack of scalability in CRISPR-Cas9 applications.

C. The inability to design sgRNA for human genomes.

D. The reduction of the PAM sequence's efficiency during editing.

Question 6 (1 mark)

A scientist is trying to use CRISPR-Cas9 to target a specific gene in eukaryotic cells. Despite designing a guide RNA complementary to the gene, the experiment fails repeatedly. Which of the following is the most likely explanation, and how should the scientist proceed?

A. The guide RNA is defective; the scientist should use a random RNA sequence to improve binding.

B. The PAM sequence near the target DNA is missing; the scientist should redesign the target sequence to include an adjacent PAM.

C. The Cas9 enzyme is ineffective in eukaryotic cells; the scientist should use an alternative editing tool.

D. The DNA repair mechanisms are too efficient; the scientists should inhibit repair pathways entirely.

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Question 7 (1 mark)

CRISPR-Cas9 technology has been used to improve the efficiency of photosynthesis in crop plants. There are two main methods that successfully change the genome of a plant.

- Method 1 aims to disable an undesired gene in a plant, which may lead to a commercial advantage.
- Method 2 aims to insert a gene into a plant without disrupting other genes.

Which one of the following assumptions could be made about methods 1 and 2?

A. Method 1 is easier than method 2 as it only involves cutting DNA, whereas method 2 involves both cutting and pasting DNA.

B. Method 2 is faster to complete than method 1.

C. Method 1 is easier than method 2 as it involves cutting RNA, which has only one nucleic acid strand, whereas method 2 involves cutting DNA, which has two nucleic acid strands.

D. Methods 1 and 2 are equally viable for future use, but CRISPR-Cas9 technology will never be widely implemented due to the ethical concept of non-maleficence.

Question 8 (1 mark)

A major concern with the CRISPR-Cas9 system is the possibility of off-target mutations which can lead to unintended genetic alterations. Which of the following strategies could be employed to minimise off-target effects in CRISPR-Cas9 applications?

A. Increasing the length of the guide RNA (gRNA) for higher specificity.

B. Using a CRISPR-Cas9 system with a high-fidelity variant of the Cas9 enzyme.

C. Applying whole-genome sequencing to identify potential off-target sites before the procedure.

D. All of the above.

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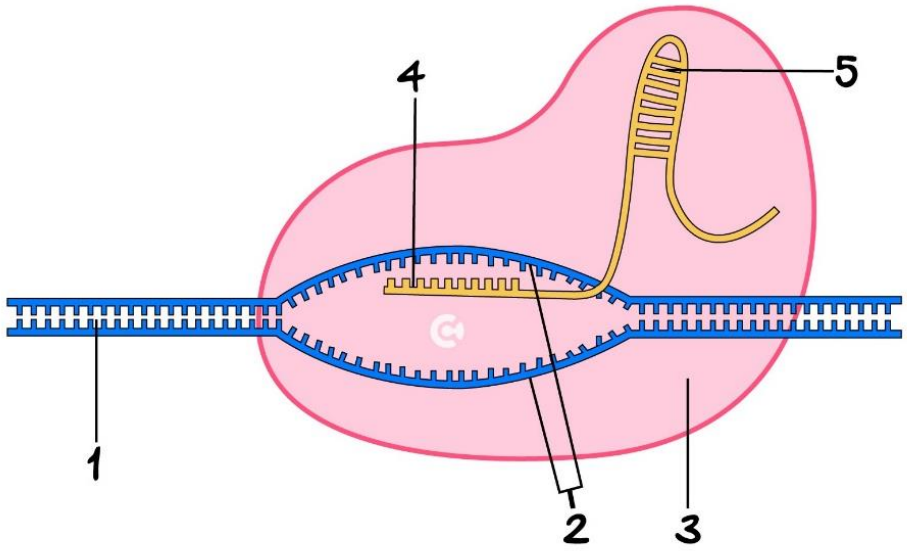
Question 9 (1 mark)

The diagram below shows the CRISPR-Cas9 complex. This is a new technology that could be superior to other gene editing technologies.



What do the components labelled **1-5** in the diagram above represent?

	1	2	3	4	5
A.	DNA	RNA	Cas9	Cutting Sites	RNA Spacer
B.	RNA	Cas9	Cutting Sites	RNA Spacer	Scaffold RNA
C.	RNA	Cutting Sites	Cas9	Scaffold RNA	RNA Spacer
D.	DNA	Cutting Sites	Cas9	RNA Spacer	Scaffold RNA

Question 10 (1 mark)

Once a specific section of DNA has been cleaved:

A. New genes can be added between the cut sections of the DNA.

B. New genes can be added to the guide RNA.

C. Another enzyme in the cytosol will join the fragments back together.

D. The CRISPR-Cas9 complex is removed from the cell.

Question 11 (1 mark)

The concept of risk minimisation and maximisation of benefit best describes which ethical principle?

A. Integrity

B. Justice

C. Respect

D. Beneficence

Question 12 (1 mark)

As the number and severity of droughts increase in Australia, scientists are developing methods to produce transgenic plants that will have an increased survival rate. Identify which one of the transgenic plants below would have an increased chance of surviving in this changing environment.

A. Rice with an introduced gene for increasing water efficiency.

B. Cotton with an introduced gene coding for herbicide resistance.

C. Squash with an introduced gene coding for mosaic virus resistance.

D. Bread wheat that was selected for its ability to grow in dry regions.

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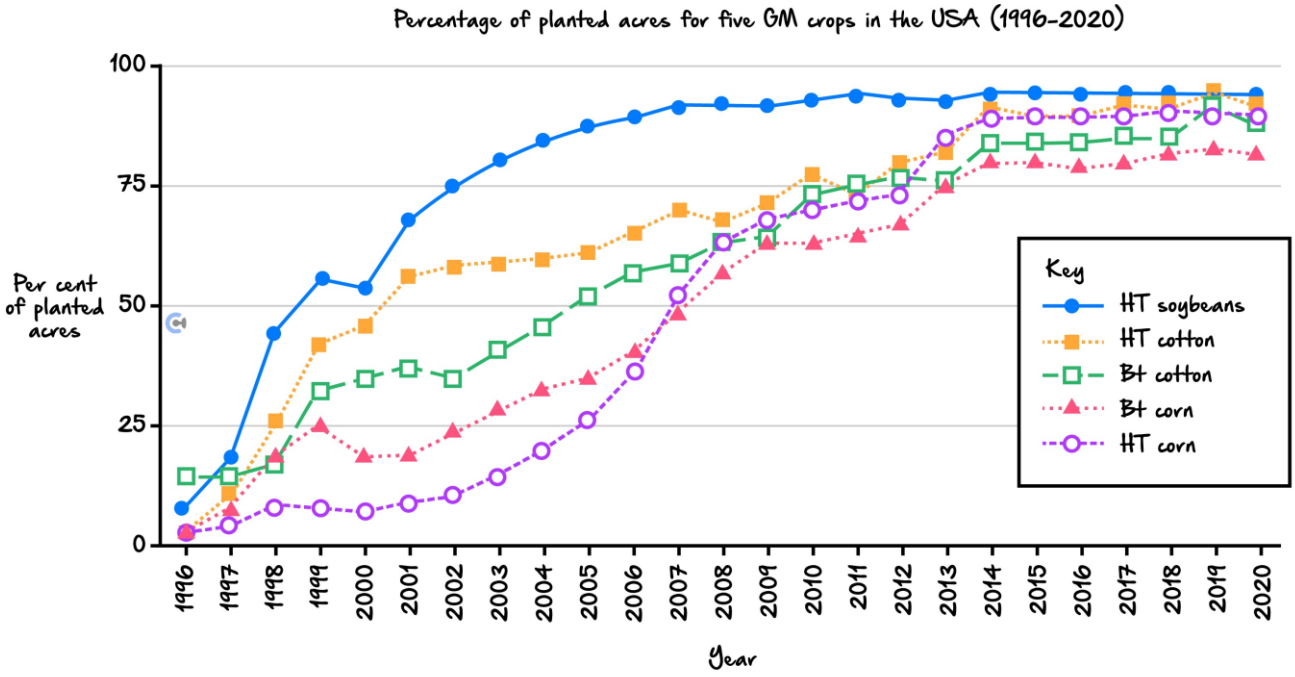
Question 13 (1 mark)

The extent to which GM crops were planted in the United States of America (USA) between 1996 and 2020 was investigated. The percentage of planted acres of GM crops out of the total planted acres of crops for five different types of crops- HT soybeans, HT cotton, Bt cotton, Bt corn and HT corn - is presented in the graph below. 'HT' indicates herbicide-tolerant varieties and 'Bt' indicates insect-resistant varieties.



Source: adapted from USDA, Economic Research Service using data from the 2002 ERS report 'Adoptaion of Bioengineeered Crops' (AER-810) for the years 1996-1999 and National Agricultural Statistics Service, (annual) June Agricultural Survey for the years 2000-2020, <www.ers.usda.gov/data-products/adoption-of-genetically-engineered-crops-in-the-us/recent-trends-in-ge-adoption.aspx>

From the graph above, it can be concluded that:

A. In 1996, the percentage of planted acres of HT cotton crops was greater than 10 per cent.

B. In 2007, the percentage of planted acres of Bt corn crops was greater than the percentage of planted acres of HT corn crops.

C. In 2013, the percentage of planted acres of Bt cotton crops and the percentage of planted acres of Bt corn crops were similar.

D. In 2020, the percentage of planted acres of HT soybean crops was the lowest that it had been for the duration of the investigation.

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Question 14 (1 mark)

Browning in apples is caused by the enzyme polyphenol oxidase (PPO). The gene that codes for PPO production is activated by stress, such as by bumping or cutting an apple. A strain of apples has been produced in which the PPO gene has been silenced, which prevents the apples from going brown.

It would be reasonable to state that these apples are:

A. The result of selective breeding.

B. Genetically modified and transgenic.

C. Genetically modified but not transgenic.

D. Transgenic but not genetically modified.

Question 15 (1 mark)

Genetically modified organisms (GMOs) that are easier to produce through farming are becoming more available around the world.

Examples of such farmed organisms include:

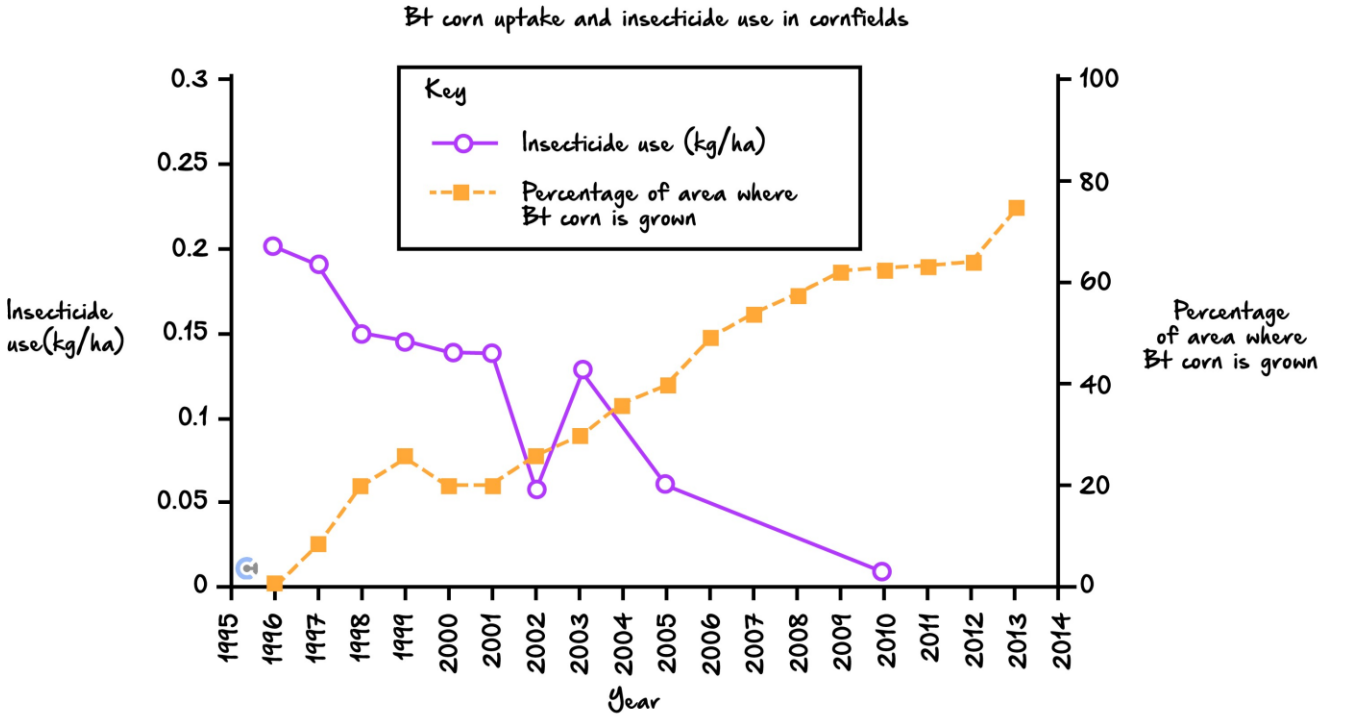
- Genetically modified corn that has an added bacterial gene, rendering the corn poisonous to some insects.
- Genetically modified potatoes that have an added RNA silencing molecule, preventing the potatoes from browning and bruising.
- Genetically modified salmon with an added growth gene from a salmon and promotor sequence from an ocean trout, increasing the salmon's growth rate.

Which row of the table shows the advantages of each GMO for farmers?

	Genetically Modified Corn	Genetically Modified Potatoes	Genetically Modified Salmon
A.	Antibiotic Resistance	Increased Size	Greater Productivity
B.	Insect Resistance	Less Food Waste	Salmon Lives Longer
C.	Lower Pesticide Use	Less Food Waste	Greater Productivity
D.	Lower Pesticide Use	Reduced Antibiotic Use	Reduced Viral Infections

Question 16 (1 mark)

Bt corn expresses a protein that acts as an insecticide.



Source: 'Smarter Pest Control', special section, Science, vol.341, 16 August 2013, p.731

Based on your knowledge and the data in the graph above, what is the benefit of using Bt corn?

A. More insecticide is used with Bt corn crops.

B. Bt corn is cheaper to produce than non-Bt corn.

C. Negative impacts on ecosystems could be reduced.

D. Fewer farmers are predicted to plant Bt corn in the future.

Question 17 (1 mark)

Which of the following best reflects the principle of beneficence in the use of CRISPR-Cas9?

A. Ensuring the benefits of CRISPR-Cas9 applications outweigh potential risks to patients.

B. Informing patients of all possible risks associated with CRISPR-Cas9 treatment.

C. Ensuring that CRISPR-Cas9 is not used for non-therapeutic purposes.

D. Preventing all possible risks associated with the use of CRISPR-Cas9.

Question 18 (1 mark)

Why is germline editing using CRISPR-Cas9 considered controversial?

A. It cannot be effectively used for therapeutic purposes.

B. Changes in the germline affect only the individual being treated.

C. It introduces changes that can be passed on to future generations, raising ethical concerns.

D. It is less effective compared to somatic gene editing techniques.

Question 19 (1 mark)

Which of the following scenarios best aligns with the ethical principle of justice in CRISPR-Cas9 applications?

A. Providing equal access to CRISPR-Cas9 treatments regardless of socioeconomic status.

B. Ensuring that only individuals with life-threatening conditions receive CRISPR-Cas9 treatment.

C. Allowing researchers to prioritise the treatment of rare diseases over common disorders.

D. Limiting CRISPR-Cas9 applications to non-human organisms to prevent human ethical dilemmas.

Space for Personal Notes

Section C: Short Answer Questions (101 Marks)

Question 20 (11 marks)

What is CRISPR, the gene editing technology that won the Chemistry Nobel prize?

CRISPR technology is adapted from a system that is naturally present in bacteria and other unicellular organisms known as archaea.

This natural system gives bacteria a form of acquired immunity. It protects them from foreign genetic elements (such as invading viruses) and lets them 'remember' these in case they reappear.

In 1987, Japanese molecular biologist Yoshizumi Ishino and his colleagues were the first to notice, in *E. coli* bacteria, unusual clusters of repeated DNA sequences interrupted by short sequences. Spanish molecular biologist Francisco Mojica and colleagues later showed similar structures were present in other organisms and proposed to call them CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats. In 2005, Mojica and other groups reported the short sequences (or 'spacers') interrupting the repeats were derived from other DNA belonging to viruses.
…
Evolutionary biologists...eventually proposed CRISPR and the associated Cas9 genes were acting as the immune mechanism.
…
The CRISPR-associated genes, Cas9, encode a protein that 'cuts' DNA. This is the active part of the defence against viruses, as it destroys the invading DNA.

In 2012, [Emmanuelle] Charpentier and [Jennifer] Doudna showed the spacers acted as markers that guided where Cas9 would make a cut in the DNA. They also showed an artificial Cas9 system could be programmed to target any DNA sequence in a lab setting.

This was a ground-breaking discovery that opened the door for CRISPR's wider applications in research.
…
Humans have altered the genomes of species for thousands of years. Initially, this was through approaches such as selective breeding.

However, genetic engineering - the direct manipulation of DNA by humans outside of breeding and mutations - has only existed since the 1970s.

CRISPR-based systems fundamentally changed this field, as they allow for genomes to be edited in living organisms cheaply, with ease and with extreme precision.

CRISPR…has great potential in food production. It can be used to improve crop quality, yield, disease resistance and herbicide resistance. Used on livestock, it can lead to better disease resistance, increased animal welfare and improved productive traits - that is, animals producing more meat, milk or high-quality wool.

A number of challenges to the technology remain, however. Some are technical, such as the risk of off-target modifications.

Source: Excerpt taken from D. Perrin, Queensland University of Technology, 'What is CRISPR, the gene editing technology that won the Chemistry Nobel prize?" The Conversation, 8 October 2020, <https://theconversation.com/what-is-crispr-the-gene-editing-technology-that-won-the-chemistry-nobel-prize-147695>

a. Identify t**wo** ways in which the CRISPR-Cas9 system is similar to the human immune system. (2 marks)

The CRISPR-Cas9 system provides bacteria with an immunological memory of specific pathogens just as the human immune system has immunological memory due to having B memory cells.
The CRISPR-Cas9 system responds to the presence of an antigen by secreting a protein that cuts the foreign DNA. The human immune system also responds to the presence of an antigen by secreting proteins.

Mark allocation: 2 marks
- 1 mark for each plausible comparison (up to 2 marks)

b. Explain how the CRISPR-Cas9 system carries out immunity functions in bacteria. (3 marks)

CRISPR arrays include a series of DNA sequences from previously encountered viruses. These short sequences of DNA are known as spacers.
When the CRISPR arrays undergo transcription, guide RNA is produced that is specific for the viral DNA that the spacers were derived from.
If the same virus invades again, the guide RNA guides the Cas molecule to the viral DNA, which is then cut by the Cas molecule.

Mark allocation: 3 marks
- 1 mark for explaining that viral DNA is incorporated as spacers in the CRISPR array.
- 1 mark for explaining that transcription of the array results in the production of guide RNA.
- 1 mark for explaining that the guide RNA guides the Cas molecule to the complementary DNA of a re-invading virus and cuts that DNA into pieces.

c. The use of CRISPR-Cas9 may result in off-target modifications. What is an off-target modification? (1 mark)

An off-target modification occurs when Cas9 cuts at unintended locations in the genome.

Mark allocation: 1 mark
- 1 mark for explaining what an off-target modification is.

d. Why would an off-target modification be a matter of concern to scientists? (1 mark)

Off-target modifications are a matter of concern because if the sequence of another gene is interrupted, then the expression of that gene may be prevented.

Mark allocation: 1 mark
- 1 mark for explaining why off-target modifications are an issue of concern.

e. Identify **one** way in which crop yields can be improved by using CRISPR-based systems. (1 mark)

Producing a strain of crops with increased disease resistance could increase the yield of that crop.

Mark allocation:* 1 *mark

- 1 mark for identifying a way in which the use of CRISPR-Cas9 technology could increase crop yield, such as by improving the quality of the crop or conferring herbicide resistance.

f. Identify **one** way in which crop yields can be improved by using CRISPR-based systems. (1 mark)

Producing a strain of sheep with higher quality wool.

Mark allocation:* 1 *mark

- 1 mark for identifying a way in which the use of CRISPR-Cas9 technology could improve productive traits in animals, such as by increasing the quantity or quality of milk or meat from cattle.

g. The article states that CRISPR-based systems fundamentally changed the field of genetic engineering because they allow for genomes to be edited in living organisms cheaply, with ease and with extreme precision.

Explain how CRISPR-based systems can be used with ease and extreme precision. (2 marks)

The use of the CRISPR array to target particular DNA sequences improves the precision of gene editing. CRISPR technology improves the ability to carry out genetic modification because it can be used to add a desirable DNA sequence into a genome, remove an undesirable sequence or modify a mutation in order to correct a DNA sequence.

Mark allocation:* 2 *marks

- 1 mark for discussing how CRISPR technology improves precision.
- 1 mark for discussing how CRISPR technology enables easier modification of DNA in living organisms.

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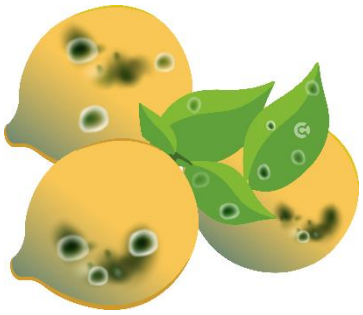
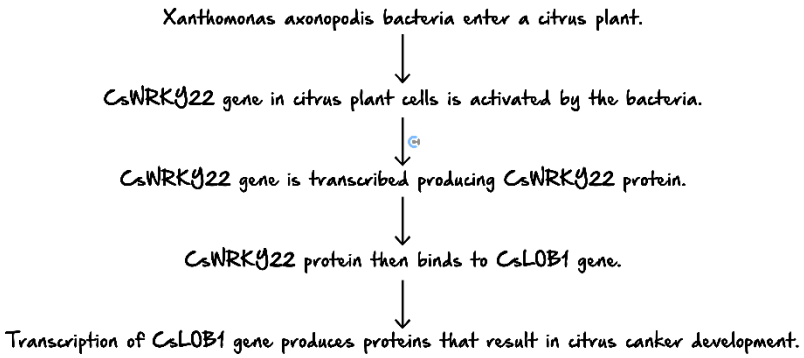
Question 21 (4 marks)

Citrus canker is a disease that affects citrus plants such as lime and orange trees, reducing fruit yield and quality by causing the development of dark spots on the fruit and leaves. It is caused by several different bacteria species, including *Xanthomonas axonopodis*. There have been several outbreaks in Australia.



Scientists have recently tried to use CRISPR-Cas9 technology to inactivate genes involved in the citrus canker pathway. This pathway for citrus canker disease is summarised below:



Using the information in the flow chart above, explain how CRISPR-Cas9 technology could be used to produce citrus-canker-resistant plants.

To produce citrus-canker-resistant plants using CRISPR-Cas9, the following steps would be taken:

1. Identify key genes: Scientists would first identify genes involved in the citrus canker disease pathway that enable bacteria to infect the plant.
2. Design guide RNA: A guide RNA (gRNA) would be designed to specifically target and direct the Cas9 enzyme to the identified genes involved in the infection process.
3. Edit the genes: The CRISPR-Cas9 system would then be used to cut the target genes, disabling their function. This could involve either knocking out the genes or altering them to make the plant resistant to bacterial infection.
4. Regenerate and test plants: The edited plant cells would be regenerated into full plants. These plants would then be tested to ensure they no longer show susceptibility to citrus canker, demonstrating resistance to the disease.

This approach enables precise modifications of the plant's genome, potentially making it resistant to citrus canker and improving fruit yield and quality.

Question 22 (6 marks)

A fungal disease called Fusarium wilt affects tomato plants, leading to wilting and eventual plant death. It is caused by the fungal pathogen *Fusarium oxysporum*, which infects the plant's vascular system. Recent research has explored the use of CRISPR-Cas9 technology to target specific genes in the tomato plant that are exploited by the fungus.

The process for Fusarium wilt progression in tomatoes is summarised below:

1. *Fusarium oxysporum* enters the plant through the roots.
2. The fungal presence activates the *FoWRKY44* gene in tomato plant cells.
3. The *FoWRKY44* gene is transcribed to produce the *FoWRKY44* protein.
4. The *FoWRKY44* protein binds to the promoter of the *FoLOB1* gene, initiating its transcription.
5. Proteins produced by the *FoLOB1* gene weaken the plant's immune response, allowing the fungus to spread.

a. Using the information in the flow chart above, explain how CRISPR-Cas9 technology could be used to produce tomato plants resistant to Fusarium wilt. (4 marks)

- sgRNA is designed to be complementary to *FoWRKY44* OR *FoLOB1*.
- sgRNA is combined with Cas9 OR guides Cas9.
- Cas9 binds to PAM and cuts the gene. As a consequence, *FoWRKY44* protein is not produced or can't bind to the *FoLOB1* gene OR *FoLOB1* gene is not transcribed, so proteins that weaken the plant's immune response are not produced.

b. Some of the tomato plants have shown a reduced ability to grow quickly in the aftermath of the editing. Propose a reason why this might be the case, explaining your reasoning. (2 marks)

- The reduced growth ability might be caused by off-target mutations introduced by CRISPR-Cas9, where genes unrelated to *FoWRKY44* or *FoLOB1* but important for growth and development were inadvertently disrupted.
- These off-target effects could interfere with critical plant processes, such as photosynthesis or nutrient uptake, leading to slower growth.

Question 23 (8 marks)

Phosphorus deficiency is a global agricultural problem that affects crop yields, particularly in maize plants. Scientists have used CRISPR-Cas9 to edit maize genomes by knocking out a gene called *PHO2*. This gene encodes a protein that reduces phosphorus uptake by degrading phosphate transporters in the roots. Knocking out *PHO2* increases phosphorus absorption and boosts plant growth.

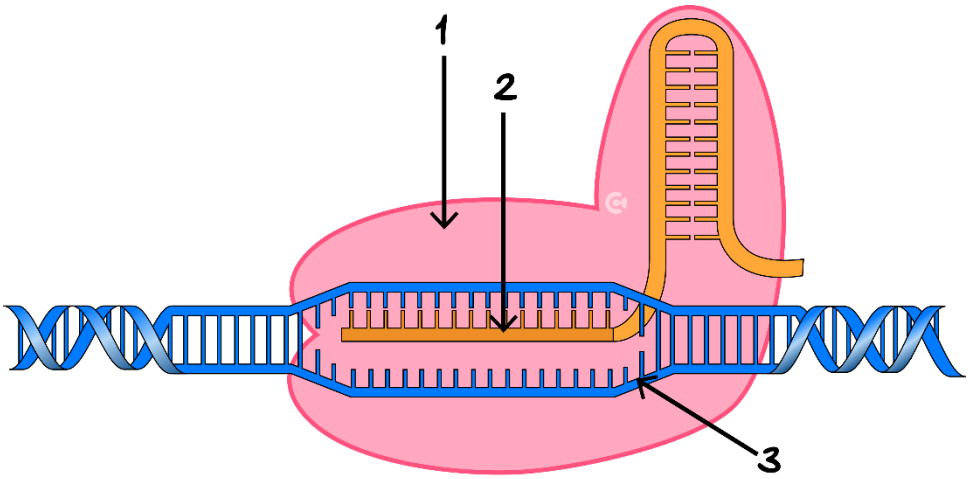
The CRISPR-Cas9 complex was used to knock out the *PHO2* gene in maize plants, producing genome-edited (GE) maize plants with enhanced phosphorus uptake.

a. Explain why maize plants edited using CRISPR-Cas9 to knock out the *PHO2* gene may be advantageous over transgenic methods for phosphorus efficiency. (2 marks)

CRISPR-Cas9 edited maize plants do not have a gene from another species inserted into the genome.
This reduces unknown harmful effects on people OR reduces the risk of environmental issues OR improves public perception/increases adoption in agriculture.

b. Phosphorus availability in soils is often limited due to its fixation in forms that are not bioavailable to plants. *PHO2* encodes a protein that degrades phosphate transporters, reducing phosphorus uptake. CRISPR-Cas9 was used to knock out *PHO2*, leading to improved root phosphorus absorption.

Refer to the diagram of the CRISPR-Cas9 complex below.



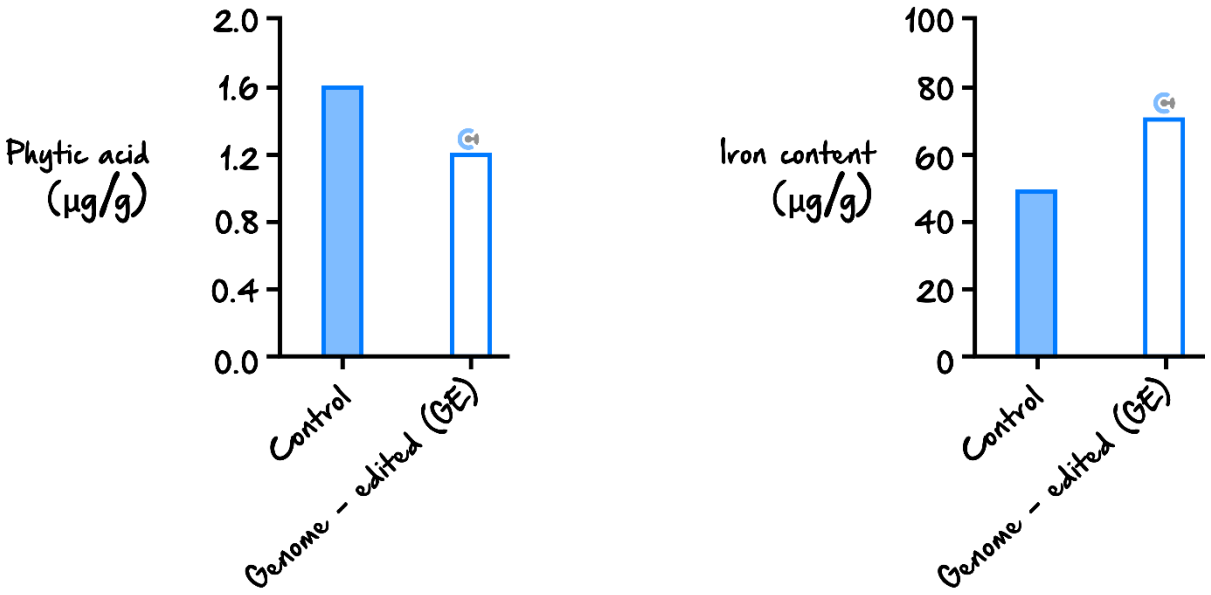
i. Which arrow, 1, 2, or 3, correctly points to the DNA strand being cut by Cas9? (1 mark)

Arrow 3

ii. Describe how Cas9 cuts the DNA at the targeted location and what role the sgRNA plays in this process. (2 marks)

sgRNA is a complementary sequence to the target DNA and guides Cas9 endonuclease to where to cut the DNA, by breaking the phosphodiester bonds between nucleotides on both strands.

c. A controlled experiment was undertaken to determine the effectiveness of using the CRISPR-Cas9 complex to knock out the *PHO2* gene in maize plants. In the experiment, maize plants were grown under identical conditions, and phosphorus uptake and plant height were measured. The results are displayed in the graphs below.



i. Using the data from the graph above, compare the levels of phosphorus uptake and plant height in the control and GE maize plants. (2 marks)

The control maize plants have lower phosphorus uptake (10 mg/kg) compared to the genome-edited maize plants (25 mg/kg).
The control maize plants have a shorter plant height (50 cm) compared to the genome-edited maize plants (70 cm).

ii. Apart from experimental errors, give one reason why GE maize plants may not show a 100% increase in phosphorus uptake. (1 mark)

Apart from experimental errors, some *PHO2* gene activity may still persist due to incomplete knockout of the gene or compensatory mechanisms in the plant's genome.

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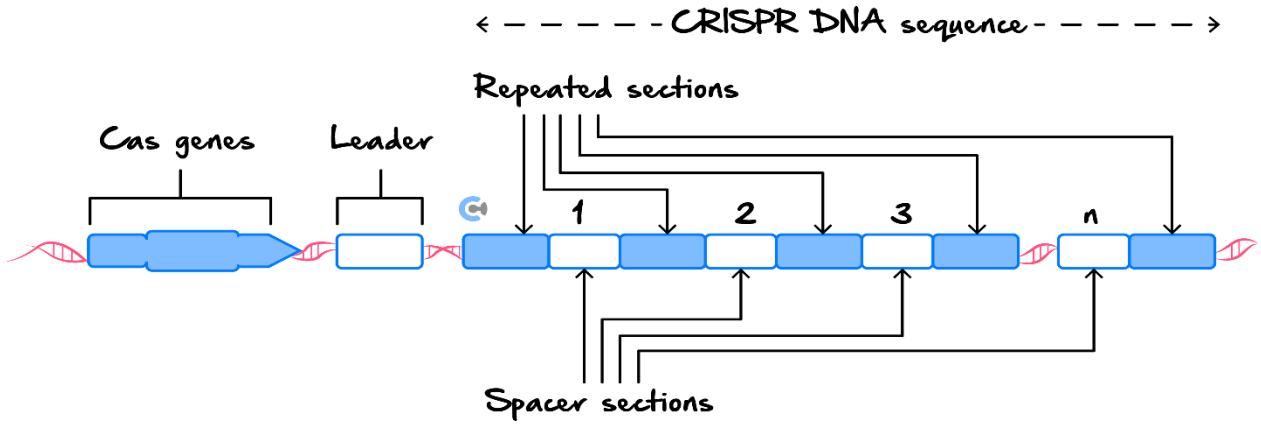
Question 24 (9 marks)

a. Prokaryotes contain a CRISPR DNA sequence, which consists of nucleotide repeated sections and spacer sections. The diagram below illustrates the positioning of these sections in part of the prokaryote circular chromosome.

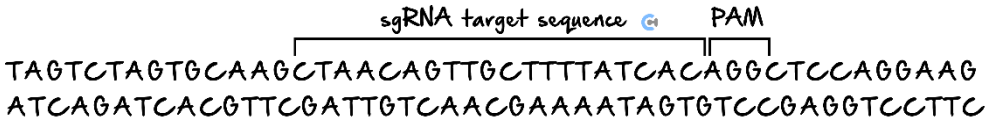


Outline the function of the CRISPR DNA sequence in prokaryotes. In your answer, state the origin of the spacers. (4 marks)

CRISPR DNA sequences function as an adaptive immune system in prokaryotes, protecting them from viruses. The spacers originate from fragments of viral DNA that the prokaryote has encountered during previous infections. These spacer sequences are transcribed into RNA, which guides the Cas proteins to recognize and target matching viral DNA during subsequent infections. The Cas proteins then cut and destroy the viral DNA, preventing the virus from replicating.

b. Sickle-cell disease and β-thalassemia are two human blood disorders. Both diseases are the result of mutations in the haemoglobin β gene. In both diseases, the ability of the red blood cells to carry oxygen is reduced. Scientists are developing a treatment that involves turning off a gene in stem cells. This will increase the oxygen-carrying capacity of the blood in people with these disorders. The scientists are using the CRISPR-Cas9 gene-editing technique to locate and edit the gene in stem cells. The gene is called *BCL11A*.

The scientists designed a single guide RNA (sgRNA) to locate a sequence of nucleotides within the *BCL11A* gene, as shown below.



Source: H Frangoul et al., 'CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia', The New England Journal of Medicine, vol. 384, no. 3, 2021, p. 254, <www.nejm.org/doi/full/doi/10.1056/NEJMoa2031054>

i. Write the nucleotide sequence that must be used on the sgRNA to locate the targeted sequence within the *BCL11A* gene. (1 mark)

The nucleotide sequence on the sgRNA must be complementary to the target sequence within the BCL11A gene. Therefore, the sgRNA sequence would be:
5'-AAUGGCUUCUCUUGACAGCUC-3'

ii. Describe the function of the PAM sequence adjacent to the sgRNA target sequence. (2 marks)

The PAM (Protospacer Adjacent Motif) sequence is essential for the CRISPR-Cas9 gene-editing system to function. It serves the following purposes:

1. Recognition and Binding: The PAM sequence, typically "NGG" (where N can be any nucleotide), is recognized by the Cas9 protein, allowing it to bind to the target DNA. Without the PAM sequence, Cas9 cannot bind and cut the DNA.
2. Cas9 Activation: The presence of the PAM sequence next to the sgRNA-targeted DNA sequence activates the Cas9 enzyme to make a double-stranded break in the DNA at the correct location, enabling gene editing.

c. Scientists have successfully edited the *BCL11A* gene. Initially, two patients were treated. Some of each patient's stem cells were extracted and then genetically modified. Each patient then had their own cells returned to them. Both responded well, with significant and sustained increases recorded in their haemoglobin levels. The two patients no longer needed blood transfusions to survive. However, both patients had some adverse reactions. The scientists have since treated another 75 patients.

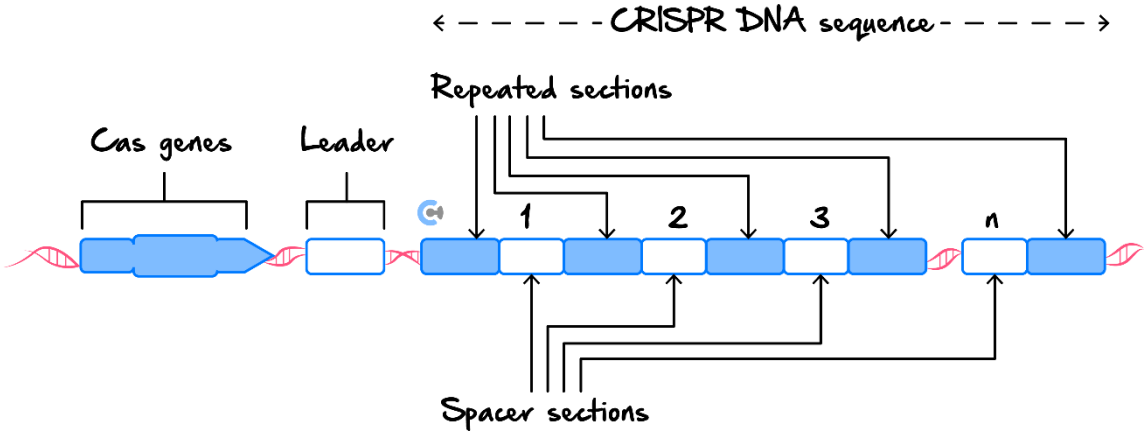
Identify an ethical concept that the scientists would have needed to consider before proceeding with the treatment of the additional 75 patients. How would they justify the continuation of the treatment in new patients? (2 marks)

Ethical concept: Safety
Justification for continuation: The scientists would justify treating new patients by demonstrating that the initial results showed significant health improvements, with the benefits outweighing the adverse reactions, and further testing would help assess and minimize risks

Space for Personal Notes

Question 25 (9 marks)

a. Prokaryotes contain a CRISPR DNA sequence, which consists of nucleotide repeated sections and spacer sections. The diagram below illustrates the positioning of these sections in part of the prokaryote circular chromosome.

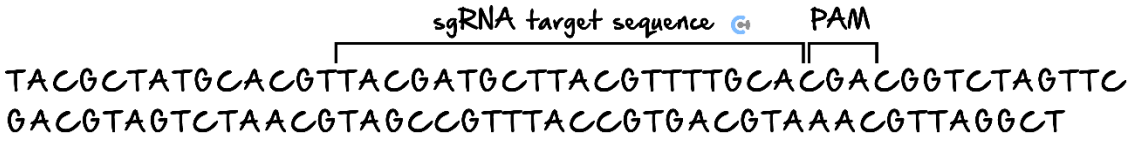


Outline the function of the CRISPR DNA sequence in prokaryotes. In your answer, state the origin of the spacers. (4 marks)

The CRISPR array provides prokaryotes with a memory of prior viral infections. The origin of the spacers is the viral DNA from these prior viral infections or bacterial plasmids.
If a prokaryote is reinfected with the same virus, then guide RNA (gRNA) is produced and attaches to complementary viral DNA sequences, or gRNA directs Cas9 endonuclease to cut viral DNA, preventing it from infecting the prokaryote.

b. Cystic fibrosis is a genetic disorder caused by mutations in the *CFTR* gene, which leads to the production of a faulty protein responsible for chloride ion transport in cells. Scientists are developing a treatment that uses CRISPR-Cas9 to correct a common mutation, Δ*F*508, in the *CFTR* gene in lung epithelial cells. This treatment aims to restore proper chloride ion transport and reduce the symptoms of cystic fibrosis.

The scientists designed a single guide RNA (sgRNA) to locate and edit the mutated sequence within the *CFTR* gene, as shown below.



i. Write the nucleotide sequence that must be used on the sgRNA to locate the targeted sequence within the *CFTR* gene. (1 mark)

The sgRNA sequence is:
AUGCGAUACGUGCAAUCGAUGGAAUGGCAAAACUGCUGCCAGAUCAAG.

ii. Describe the function of the PAM sequence adjacent to the sgRNA target sequence. (2 marks)

The PAM sequence acts as the binding site for the Cas9 endonuclease and serves as the target for designing sgRNA upstream of this sequence or allowing Cas9 to cut the DNA sequence.

c. Scientists have successfully corrected the *CFTR* gene. Initially, two patients were treated. Some of each patient's lung epithelial cells were extracted and then genetically modified. Each patient then had their own cells returned to them. Both responded well, with improved lung function and a reduction in cystic fibrosis symptoms. The two patients no longer required intensive respiratory treatments. However, both patients experienced mild inflammatory side effects. The scientists have since treated another 75 patients.

Identify an ethical concept that the scientists would have needed to consider before proceeding with the treatment of the additional 75 patients. How would they justify the continuation of the treatment in new patients? (2 marks)

Examples of ethical concepts and their justification for continuing treatment in new patients include:

- **Beneficence:** The benefits of the (genetically modified epithelial cell) treatment outweigh the adverse effects.
- **Integrity:** Reporting all results so patients have the choice of receiving the (genetically modified epithelial cell) treatment or not.
- **Respect:** Informing patients of the possibilities of any adverse effects of receiving the (genetically modified epithelial cell) treatment.
- **Non-maleficence:** Ensuring adverse effects associated with receiving the (genetically modified epithelial cell) treatment are minimised.

Question 26 (8 marks)

The CRISPR-Cas9 complex is a gene-editing technology used in a variety of contexts.

a. Describe the role of the CRISPR-Cas9 complex in a bacterium. (2 marks)

The CRISPR-Cas9 complex allows the bacterium to cut sections of foreign viral DNA and incorporate them into its CRISPR gene.

Thus, the bacterium can 'remember' the bacteriophage so that if the bacterium is infected again, it will be able to identify and destroy the foreign DNA.

b. Describe the function of a spacer in a bacterium. (1 mark)

The spacer is complementary to a section of the viral DNA.

Recent clinical trials have used the CRISPR-Cas9 complex to treat sickle cell anaemia in adults. In the treatment, the bone marrow stem cells of an individual with sickle cell anaemia are extracted and mixed with a CRISPR-Cas9 complex that is designed to disrupt the adult haemoglobin gene. Once the gene is disrupted, the stem cells are placed back into the individual. The cells then differentiate and express foetal haemoglobin, which alleviates the symptoms of sickle cell anaemia.

c. Describe how a CRISPR-Cas9 complex could target and disrupt the adult haemoglobin gene. (3 marks)

A strand of guide RNA is constructed with a spacer section that is complementary to the adult haemoglobin gene.

The single guide RNA is added to a CRISPR-Cas9 complex, which is then added to the extracted stem cells. The guide RNA binds to the haemoglobin gene in a complementary fashion.

The DNA in the haemoglobin gene is then cut at the location the guide RNA is bound to. When the DNA is repaired with other nucleotides, the DNA sequence is altered and the gene is thus disrupted.

d. As CRISPR-Cas9 technology becomes increasingly common, questions have been raised about its use in medical treatments for babies. A survey consisting of five questions about the use of CRISPR-Cas9 technology in the treatment of babies is planned to assess the general public's opinion on the topic. The questions are shown in the table below.

Question number	Question
1	Do you think the technology will only be available to the wealthy?
2	Do you think the technology will be used by some in morally unacceptable ways?
3	Do you think the technology will be used before the health effects are fully understood?
4	Do you think the technology will lead to the development of other new medical advances?
5	Do you think the technology will help people live longer and better-quality lives?

Describe the ethical concept of beneficence and identify one question in the survey that refers to beneficence. (2 marks)

The ethical concept of beneficence relates to the maximisation of benefits when undertaking a particular course of action.

Question 4 **OR** 5 relates to the additional benefits associated with this research; thus, it relates to beneficence.

Space for Personal Notes

Question 27 (10 marks)

Golden rice is a special form of rice that was developed to combat vitamin A deficiency, which is a major cause of preventable blindness in developing countries where there is not sufficient access to eggs, liver, and dairy which are all rich in vitamin A.
Given that rice is a staple food in many of these countries, scientists thought that developing a form of rice that would have increased vitamin A levels would improve this issue of vitamin A deficiency, and they achieved this by adding the PSY gene from a daffodil and the CRTI gene from a soil bacterium. This causes the rice to store beta-carotene, in the grains of rice rather than the leaves, giving them that distinctive golden colour.

a. Define the term 'GMO' and describe specifically the type of GMO golden rice that would be considered to be. (2 marks)

An organism with genetic material that has been altered using genetic engineering technology, to insert, remove, silence, or alter it. Golden rice would be considered to be a transgenic genetically modified organism, due to the fact that it contains genetic material from other species.

b. Why might it be beneficial for the rice to store the beta-carotene in the grain and not in its leaves? (2 marks)

The rice itself would have no functional benefit to storing the beta carotene in the grain.
We eat the grain of the rice and not the leaves, and the beta carotene being present in the grain means that we will ingest the majority of the beta carotene content. This increases individuals beta carotene intake from the rice, and this results in reduced vitamin A deficiency.

c. Discuss some of the biological implications that may result from the use of golden rice widely. (2 marks)

Other answers may be acceptable – Ask Aaliyan
The golden rice may cross pollinate with other crops and this may cause unintended impacts on biodiversity in the region.
The golden rice would improve the biological health of the wider population that consumes them.

d. Discuss, with reference to the principles of beneficence and justice, the ethics of introducing golden rice into widespread use across Southeast Asia. (4 marks)

Beneficence – the idea is to do the most good, that is the duty ethically speaking:

- Improving Vitamin A deficiency rates is doing the most good – hence it is ethical.
- There may be further unintended health impacts that we might yet be unaware of – this may not be in the interest of doing good and may actually cause harm.

Justice – the idea that the distribution of resources should be such that it promotes fairness and equality for all, and no group is impacted adversely of positively disproportionately compared to another.

- Ensuring widespread access to the golden rice via this project may mean that the principle of justice is upheld.
- Acting on an area of inequality – Vitamin A deficiency – is something that might improve the fairness of health outcomes somewhat.
- However, research has shown that this may divert resources away from existing initiatives which may prove to be more effective and be accessible to more people.

Question 28 (13 marks)

The development of more precise genetic editing technologies, such as CRISPR-Cas9, has brought life to the prospect that genetic editing can become popular, safe, and effective in the near future. Some visionaries have suggested that this may usher in a new era wherein human embryos can be edited for desirable traits and characteristics, as well as protecting them from potential diseases as well.

a. Describe how CRISPR-Cas9 could be applied in an experiment to edit a gene causing disease in an embryo. (3 marks)

A gene may be causing disease as it produces a protein product that is malfunctional, and needs to be prevented from doing so.

- Identify a target sequence for a cut, 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, and then the sgRNA will bind to the target DNA, and then signal the cut.
- Cell repair mechanisms will try and repair causing errors, thus silencing the gene, and preventing its expression.

b. What are some concerns regarding the usage of CRISPR-Cas9 to edit human embryos? (3 marks)

Respect! – the embryos are inherently unable to consent to any edits being made, this is not respecting their autonomy at all. Some may argue that this doesn't respect the sanctity of human life.
Beneficence! Even if there may be a benefit conferred, might be unaware of potential impacts and the viability of that benefit in the first place.
Non-maleficence! Research may be potentially harmful to the embryo itself and mav result in its loss.

c. Using a consequences-based approach, and the principles of beneficence and justice, compare and discuss the ethics of using CRISPR-Cas9 to fix known disease-causing abnormalities as opposed to "preference" edits (e.g. parents wanting blue eyes not brown.). (5 marks)

- We may not understand the consequences that much, so designer edits might be unintentionally harmful.
- Consequences might actually work out to be well (diseases being cured) in the end so things might end up being good.
- Can do a lot of good with this editing technology, will improve the quality of life, compared to designer edits.
- This technology may not be available to everyone and hence may cause some class issues and class dynamics, with those with a lower socio economic bakcground being unable to access these conditions and ultimately being left behind and this may entrench that genetically.
- Difficult to tell how some edits may have a potential benefit – may unneccesarily promote a procedure.

Currently, despite the promise, there are still concerns over the efficacy of the CRISPR-Cas9 treatments, and multiple rounds of trials are required before approvals can be granted.

d. With reference to the principle of non-maleficence, discuss the need for extensive clinical trials before making this technology available to a wider public. (2 marks)

Extensive clinical trials are in place during the approvals process of medical treatments, to ensure that there are no undue harms in the treatment. This is designed to ensure that the principle of non-maleficence is upheld, which indicates that harms should be minimised. Some harms may be acceptable, as long as they are not disproportionate to the benefits, in this case, the potential of becoming a tutoring superhero.

Question 29 (9 marks)

Bt cotton is a type of cotton that contains two genes from a soil bacterium, *Bacillus thuringiensis*, enabling it to produce insect-resistant proteins. Australian farmers of Bt cotton use only 15% of the quantity of the insecticide that was once needed to protect their cotton crops. However, Bt cotton is not as resistant to the main insect pest of cotton crops, *Helicoverpa*, as it has been in the past. In Australia, Bt cotton is picked by machine, but in India, it is picked by hand. Workers in India have developed skin allergies, which have been attributed to Bt cotton proteins. Traditionally, farmers have saved money by keeping seeds from one year's crop to plant the following year. However, it is illegal for farmers to keep Bt cotton seeds because these seeds have been declared the legal property of the company Monsanto. Every year, cotton farmers must buy more seeds from Monsanto.

a. Is Bt cotton transgenic or cisgenic? Justify your answer. (2 marks)

Bt Cotton is a genetically modified organism , considered to be transgenic. Transgenic – as it involves genetical material being inserted from a foreign species, in this case a soil bacterium.

b. Discuss the social implications of the use of Bt cotton. (2 marks)

- Farmers spend less money and effort protecting their plants which improves costs, and their livelihoods as well. (they make more profit) – POSITIVE.

However,

- The legality of re using the seeds (given that farmers must buy seeds every year) may cause stress and damage to the farmers – NEGATIVE.

Note: There are a number of other acceptable answers.

c. How might Bt cotton result in increased crop yields? (1 mark)

Fewer plants are dying due to the resistance provided by the gene, resulting in more plants surviving to maturity/ripeness, allowing more plants to be harvested per unit of area – thus resulting in an increase in crop yields.

d. Discuss, using a consequences-based approach and applying the principle of justice, the ethics of this model of Bt cotton. (4 marks)

Justice – the principle that there should be fair consideration of competing claims, that the benefits of an action should be distributed equally and fairly.

Buying new seeds every time is this fair?

- Providing an advantage to those who have had them and those who do not , which entrenches inequality. This is against the principle of justice.
- This company may also establish full control over the market for such seeds, making them a neccesity and potentially inaccessible, thus establishing further unfairness and inequality.

Consequences Based Approach – judging whether an action should be undertaken or not by focusing on its consequences.

Impacts of using Bt cotton:

- Improves crop yields which helps people get more food.
- Might have unintended biodiversity impacts.

Space for Personal Notes

Question 30 (14 marks)

The Tasmanian Tiger, or the Thylacine, was a marsupial that lived in Australia, before it went extinct after the arrival of European settlers, with the last one dying in captivity in 1931.

Recently, there has been talk about using DNA manipulation technologies to "revive" the thylacine and bring it back, by editing it with current species still present today. Researchers claim that this would be 'de-extinction' and will bring the thylacine back to life, but in reality, an exact replica of the original animal will not be created, rather, it will be a hybrid creature.

There are a number of concerns that have been raised over this project.

The researchers claim that this is a step in reversing the damage done ecologically by the settlers, and bringing the ecosystem back to balance, whereas critics claim that this may actually end up having adverse impacts on an ecosystem that has adapted to modern challenges.

Further to this, concerns have been raised over how the embryos will be born, whether through a surrogate or otherwise, and the welfare of the thylacines after birth.

a. Describe some of the social and biological implications of this plan. (3 marks)

Social Implications:

- This program may undermine existing conservation efforts which are economically more viable than this plan.
- This may set a wider precedent regarding our treatment of the environment and wildlife, as people may now consider these events to be easily reversible.

Biological Implications:

- The edited animals may have unintended impacts on ecological balance, especially as the ecosystem has adapted to life without the thylacine.
- The introduction of the thylacine may cause biodiversity issues as they may have genetic challenges we are unaware of as we have edited them.
- The animals may help the interactions of existing wildlife by returning situations similar to a time before human intervention.

b. Would these be considered GMOs, and if so which type? Explain. (2 marks)

Yes, it would be considered a genetically modified organism, as it contains alterations of its genetic material from other sources. In this case, it would be considered transgenic, as it involves genetical material being inserted from a foreign species, in this case a soil bacterium.

c. With reference to the principles of beneficence and respect, discuss the ethics of this plan of reviving the thylacine. (4 marks)

Beneficence – the principle that we should strive to do the most 'good' or confer the most benefit.

- Bringing them back may result in a positive impact of biodiversity and the environment, with an affirmative step to overcome our errors before, better for beneficence.
- Bringing them back may cause unintended environmental impacts, which would harm the environment going against the principle of beneficence.
- Genetically, there may be uninteded consequences from these edits, resulting in negative impacts further down the line.
- May spark further research into this area, which could increase the resources availble for conservation efforts.

Respect – the principle that all living things have an intrinsic value and that we should respect their rights.

- The welfare of other animals is being respected, as they are not being forced into being surrogates.
- Welfare of the new creatures – will they be able to survive properly in the wild without any similar creatures?
- Respect for the environment, trying to reverse the damages created by humans.
- Survivability of the new animals in the new environment.
- Respect for the welfare of the exisiting environment, given the desire to introduce a brand new species, which may be harmful.

d. Why it might be problematic to claim that this is a 'de-extinction' of the thylacine? (3 marks)

This is problematic when considering the ethical concept of integrity which requires all information to be reported honestly and fairly.
The new species that is created will be genetically distinct from the actual original thylacine, whereas the term de-extinction implies it will be genetically identical.
Hence, calling it de-extinction is misleading, and is problematic when considering the principle of integrity.

e. Explain the difference between the virtues-based and duty-based approaches to ethics. (2 marks)

- A duty/rule-based approach is concerned with how people act (the means) and places central importance on the idea that people have a duty to act in a particular way, and/or that certain ethical rules must be followed, regardless of the consequences that may be produced.
- On the other hand, a virtues-based approach is person, rather than action-based. Consideration is given to the virtue or moral character of the person carrying out the action, providing guidance about the characteristics and behaviours a good person would seek to achieve to then be able to act in the right way.

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