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VCE Biology ¾
CRISPR-Cas9 & Bioethics [1.6]

Workbook Solutions

Outline:

Т			-
An Introduction to CRISPR-Cas9 ➤ Overview of CRISPR-Cas9 ➤ Viral Life Cycle	Pg 3-6	Applications of CRISPR-Cas9 ➤ Introduction to Genetic Engineering	Pg 17-19
 CRISPR-Cas9 in Bacteria Exposure Expression Extermination 	Pg 7-11	 Bioethics in VCE Approaches to Bioethics Bioethical Concepts General Ethical Scenarios Applying Ethics to GMOs 	Pg 20-35
CRISPR-Cas9 in Gene Editing	Pg 12-16	Applying Ethics to CRISPR-Cas9	

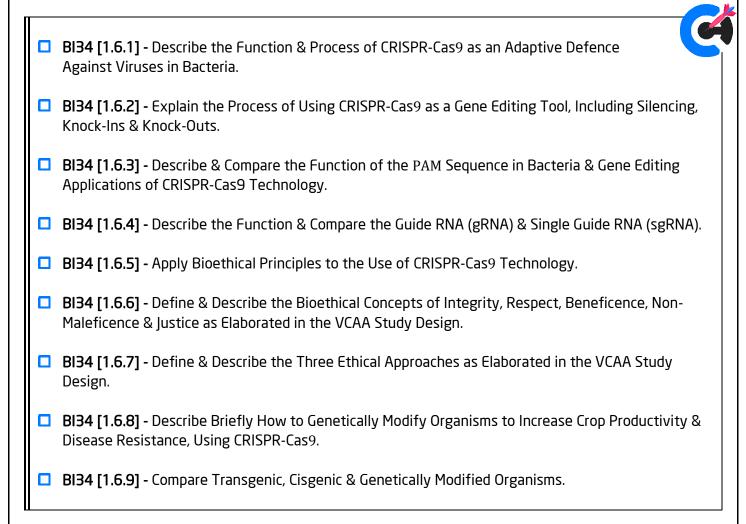
Study Design: CRISPR-Cas9 and Recombinant Plasmids



The function of CRISPR-Cas9 in bacteria and the application of this function in editing an organism's genome.



Learning Objectives:





Section A: An Introduction to CRISPR-Cas9

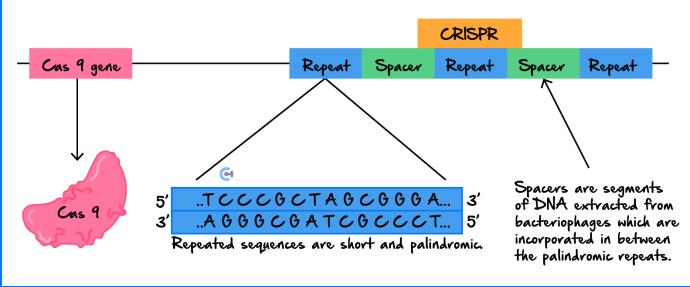
Sub-Section: Overview of CRISPR-Cas9



How do bacteria protect themselves against infection?

What is CRISPR-Cas9?

- CRISPR-Cas9 is a naturally occurring immune defence mechanism that is found within ____ bacteria to protect themselves against _____ bacteriophage ____ (viruses that attack bacteria specifically).
 - For a bacterial cell, viral infection means ______ death _____, so this mechanism has evolved to prolong its survival.
 - It "stores" some viral DNA to remember it, and uses it to recognise the viral DNA upon reexposure. Deactivates DNA by ____ cutting it ____.
- We have adopted this mechanism in bacteria, to use it for our own purposes of _ gene editing ____
- Clustered Regularly Interspaced Palindromic Repeats





Analogy: Surveillance and Security



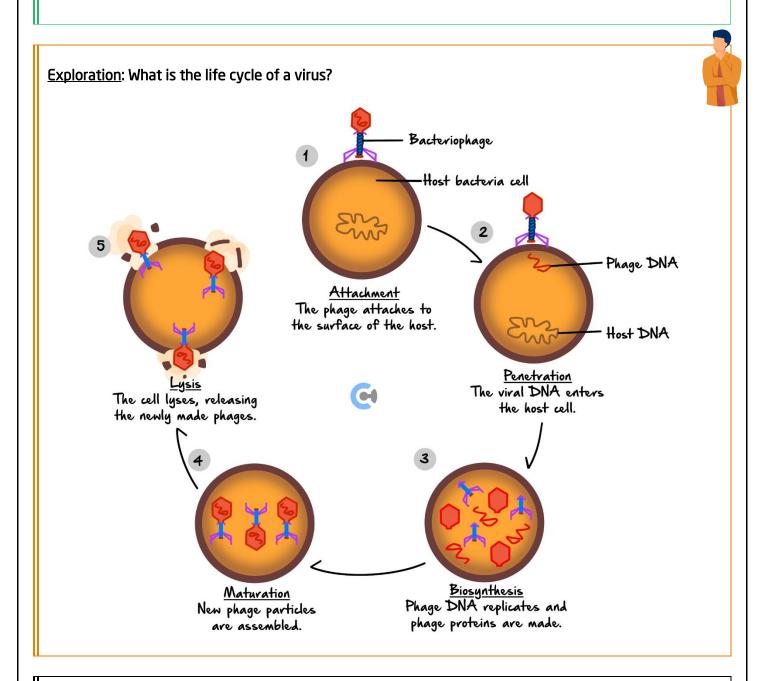




Sub-Section: Viral Life Cycle



How do viruses infect bacteria?





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✓ Overview:

- CRISPR-Cas9 is a revolutionary tool derived from bacteria's natural defence mechanism against viruses (bacteriophages).
- It stands for Clustered Regularly Interspaced Short Palindromic Repeats.
- G Function: Allows for precise and efficient gene editing by targeting specific DNA sequences.

✓ Analogy:

lt works like a surveillance system: bacteria "store" viral DNA to recognise and neutralise future invasions.

Key Features:

- Cas9: A programmable enzyme (endonuclease) that cuts DNA.
- Guide RNA (gRNA): Guides Cas9 to the specific target DNA via complementary base pairing.
- PAM (Protospacer Adjacent Motif): A short DNA sequence that enables Cas9 to bind and cut at the correct site.

Applications:

Gene editing technology leverages CRISPR-Cas9's ability to precisely cut DNA, enabling modifications like silencing, knock-ins, and knock-outs.

A

How can we stop this?



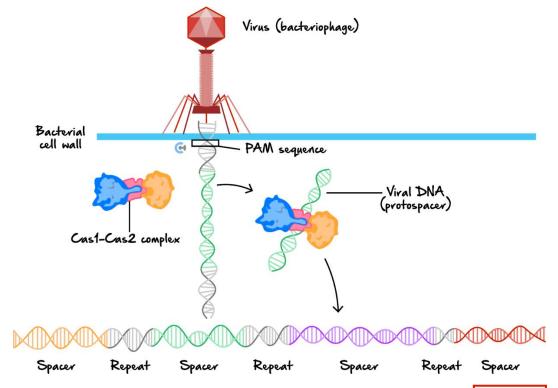
Section B: CRISPR-Cas9 in Bacteria

We can divide this into 3 key stages...

Sub-Section: Exposure

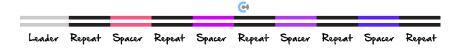


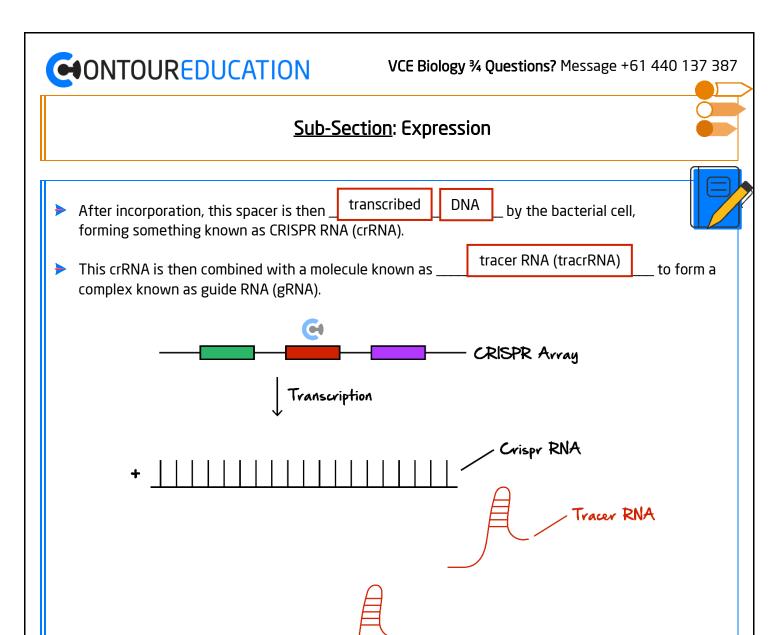
- A bacteriophage will attach to a bacterial cell and inject it's _____ DNA _____ into the cell.
- The cell will recognise this as foreign, and some _____ endonucleases ___ such as Cas1 and Cas2 will cut out a section of the viral DNA known as a _____ protospacer ____.
 - The enzymes will cut out the protospacer into about 30 nucleotides big, and it MUST be next to the PAM sequence, which is ___ not included in the actual spacer itself ______.
 - PAM stands for _____ protospacer adjacent motif



This protospacer is then incorporated into the bacterial chromosome at the ____ CRISPR ___ locus.

Think of the name for it!







= Guide RNA

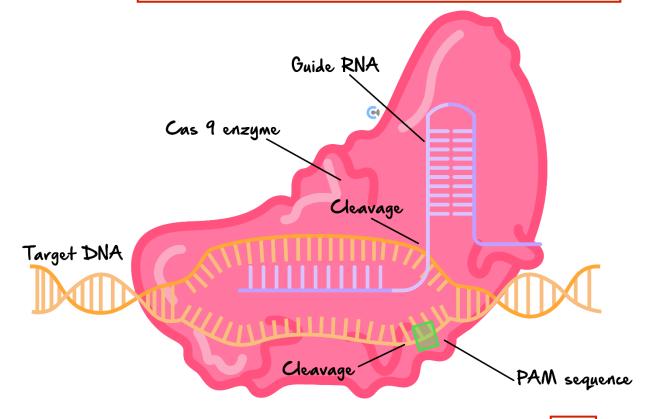


Sub-Section: Extermination



The gRNA will bind to the Cas9 enzyme to form the CRISPR-Cas9 complex, which will float around the cell _____ trying to find the sequence of viral DNA complementary to the gRNA





Once a match is found- via complementary base pairing, the Cas9 is activated to ____ cut ___ the DNA, therefore inactivating it.

REMINDER: Extermination is when the virus tries to infect the cell once more!





Sample Response: Describe the Process of CRISPR-Cas9 in Bacteria



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

Discussion: What is the significance of the PAM?



Recognition of self vs non-self in bacterial applications:

HAS TO BE PRESENT TO ENSURE THAT CAS9 CAN CUT.

Can compare it to a LANDING PAD- it will bind THEN check the main sequence.

Efficiency- it won't have to bind to any random DNA.

Discussion: What happens to DNA after it has been cut in a cell?



The cell tries to repair the actual DNA using its own mechanisms, but this can introduce errors that result in inactivation.

Active Recall: What are the steps in CRISPR-Cas9 function in bacteria?







Active Recall: What is the function of the guide RNA?



Key Takeaways



✓ Function:

- CRISPR-Cas9 acts as an adaptive immune system in bacteria to protect against viruses.
- Viral DNA is recognised, cut, and incorporated into the bacterial genome at the CRISPR locus as a "spacer."
- On subsequent infections, bacteria transcribe spacers into RNA, which combines with Cas9 to seek and destroy matching viral DNA.

✓ Process:

- 1. **Exposure:** A bacteriophage injects its DNA into a bacterial cell.
 - Endonucleases cut a protospacer (a small viral DNA fragment) adjacent to a PAM sequence.
- **2. Incorporation:** The protospacer is integrated into the bacterial genome.
- **3. Expression:** The spacer is transcribed into crRNA and combines with tracrRNA to form guide RNA (gRNA).
- **4. Extermination:** gRNA directs Cas9 to recognise and cut complementary viral DNA, inactivating the virus.

☑ Role of PAM:

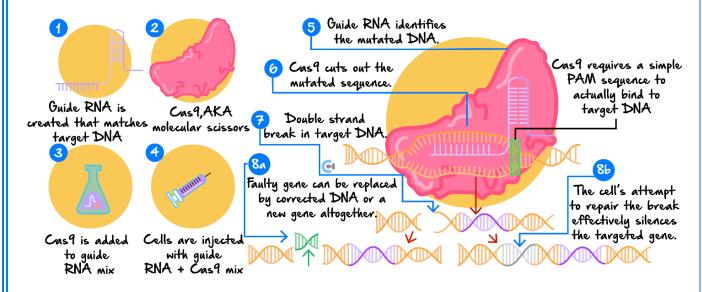
- PAM ensures that Cas9 binds to and cuts only the foreign DNA, distinguishing self from non-self-DNA. It also ensures that the Cas9 will only bind to DNA and unwind it for detecting a match where it is possible.
- This system is highly specific and efficient, making it adaptable for gene editing.



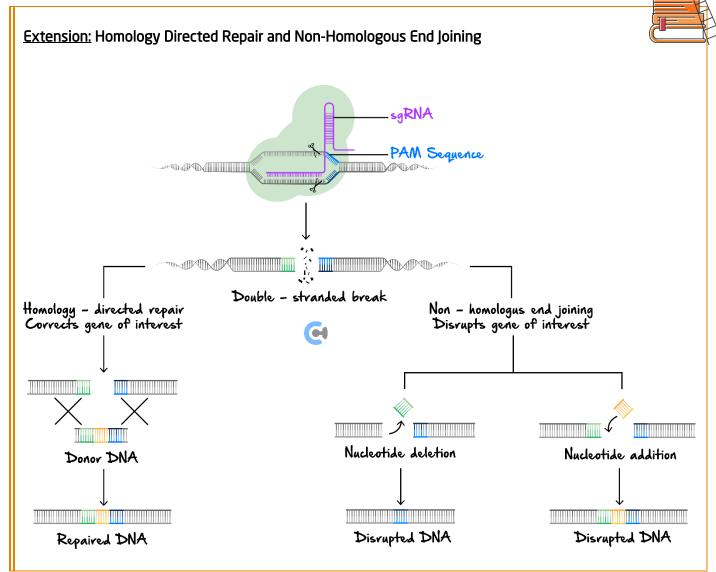
Section C: CRISPR-Cas9 in Gene Editing

CRISPR - Cas9 in Gene Editing

- Using this principle of CRISPR, we can essentially get a targeted endonuclease.
 - Cas9 cuts wherever there is a PAM and a complementary DNA sequence to its gRNA-paired molecule.
 - We can alter the PAM and create our own gRNA called _____ single ____ guide RNA (sgRNA).
- Gene edits occur when Cas9 cuts a gene at the target sequence and:
 - Knock-out/silence a gene- cell repair mechanisms will induce mutations to silence the gene.
 - Make edits to a gene.
 - Insert a gene.







REMINDERS

- The steps for the usage of CRISPR in bacterial cells and in actual experiments are DIFFERENT. CRISPR gene edits generally don't involve any virus, and the gRNA is single.
- The PAM is unique to each organism and can be altered for research purposes in experiments.
- In experiments, it can work to reduce or enhance/create a new function in genes.



Sample Response: CRISPR-Cas9 in Gene Editing



- 1. Identify a target sequence for a cut, and develop a synthetic guide RNA (sgRNA) complementary to it.
- 2. Combine this with the Cas9 enzyme, with a PAM suitable to the target.
- **3.** Inject this into a target cell, then the sgRNA will bind to the target DNA, and then signal the cut.
- **4.** Cell repair mechanisms will try and repair causing errors, or will repair using the gene you want to insert.

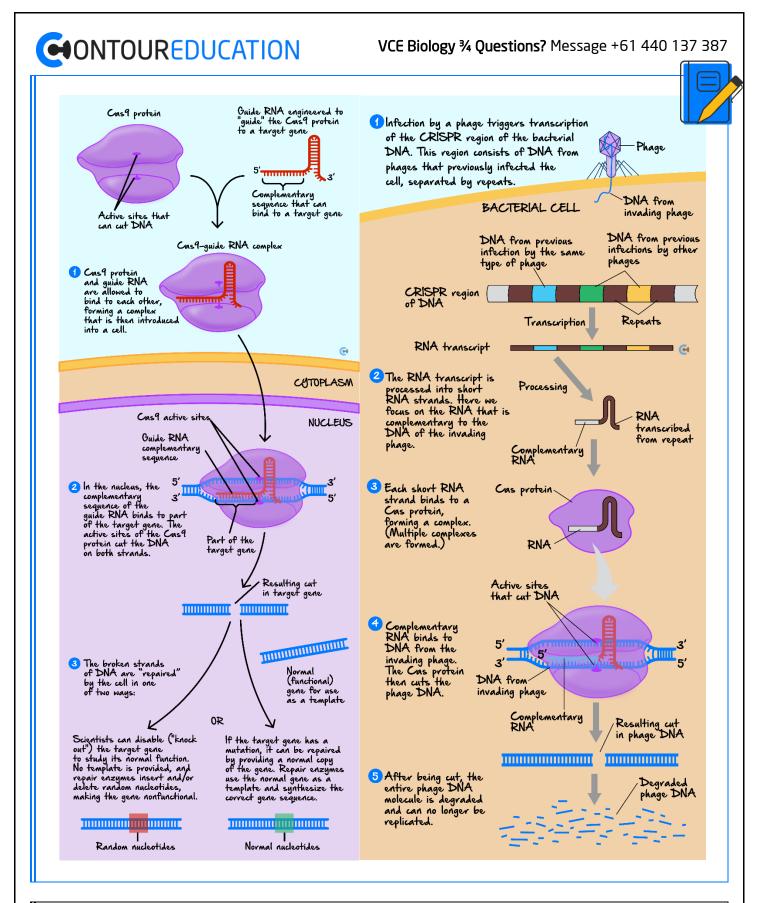
REMINDER: Ensure to relate the specific edit, and TYPE of edit to the scenario!



What does VCAA say?



- Students should understand that CRISPR-Cas9 functions as a primitive adaptive immune system in bacteria. They should know that CRISPR is a specialised sequence of DNA with two important features:
 - Nucleotide repeats and spacers: The spacers are segments of DNA cut from invading viruses (bacteriophages) that allow the bacteria to recognise the same virus in the event of subsequent invasions.
 - Students should recognise that Cas9 is an endonuclease associated with CRISPR and acts like a pair of molecular scissors capable of precisely cutting both strands of DNA. They should understand that Cas9 will only cut the target sequence if it recognises a very short nucleotide sequence adjacent to the target spacer called a protospacer adjacent motif (PAM) sequence. The PAM sequence plays an essential role in distinguishing self from non-self. Students should understand that gene editing in eukaryotes using CRISPR-Cas9 technologies rely on the use of Cas9 protein and single guide RNA (sgRNA) molecules.
 - Knowledge of other Cas enzymes and the other forms of RNA involved is not required. Students should understand that the CRISPR-Cas9 system is an easy and cost-effective way of editing an organism's genome, with many applications. Increasing photosynthetic efficiencies and crop yields are the specific examples that teachers should use to demonstrate the use and application of CRISPR-Cas9 technologies.
 - However, students should also be able to apply their understanding to other areas of the study design and to other unfamiliar situations and contexts.







Key Takeaways



✓ Overview:

- CRISPR-Cas9 has been adapted as a powerful and versatile tool for editing genomes in eukaryotic cells.
- The system uses the principles of bacterial immunity but has been optimised to make precise cuts in specific DNA sequences within any organism.

✓ How it Works:

1. Target Sequence Identification:

- ✓ A target DNA sequence is selected for editing, such as a gene responsible for a disease.
- Researchers design synthetic **single guide RNA (sgRNA)**, which combines the targeting properties of crRNA and tracrRNA into one molecule.

2. CRISPR-Cas9 Complex Formation:

✓ sgRNA binds to the Cas9 enzyme, forming the CRISPR-Cas9 complex.

3. DNA Binding:

- ☑ The complex locates the target DNA by matching the sgRNA sequence to the complementary DNA sequence.
- A **PAM sequence** (e.g., NGG) adjacent to the target ensures proper binding and initiates the cutting process.

4. DNA Cutting:

Cas9 creates a double-strand break at the target site.

5. Repair Mechanisms:

- ▼ The cell's natural repair mechanisms are activated:
 - Non-Homologous End Joining (NHEJ): Repairs the break but often introduces errors (e.g., insertions or deletions), which can disable the gene (gene knock-out).
 - Homology-Directed Repair (HDR): Uses a provided DNA template to repair the break precisely, allowing the insertion of new genetic material (gene knock-in).





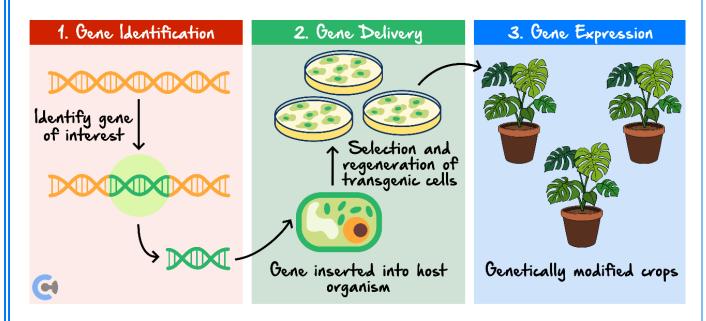
Section D: Applications of CRISPR-Cas9

Sub-Section: Introduction to Genetic Engineering



Overview

- Definition
- Genetic engineering is the alteration of a genome via genetic recombination- taking DNA from foreign sources and introducing it to an organism.
 - Genes can be added, removed, silenced or edited to alter them.
 - We can use CRISPR-Cas9 to achieve this, but this is not the only method.
- GMO? Genetically Modified Organism.
- Transgenic organisms _ are a type of GMO, wherein there are genes from foreign species introduced
- Cisgenic organisms ____ are when there is foreign DNA introduced to an individual, but it is from another individual of the same species



- ➤ We use them to improve crop productivity- improving their features including examples, such as golden rice.
- We use them to improve disease resistance, resulting in fewer crop deaths.



Active Recall: Describe how CRISPR-Cas9 could be used to edit these plants!



Discussion: How could this be assessed in a SAC or Exam?



Key Takeaways

1. Gene Editing

CRISPR-Cas9 enables targeted genetic modifications for various purposes:

- Gene Silencing (Knock-Outs):
 - ☑ Cas9 introduces a double-strand break at a target DNA sequence.
 - ✓ The cell repairs the break via non-homologous end joining (NHE), which often introduces mutations, disabling the gene.
 - ☑ Example: Disabling genes linked to diseases like cancer or Huntington's disease.

Gene Knock-Ins:

- Homology-directed repair (HDR) uses an introduced template to insert new DNA sequences.
- Example: Adding a functional copy of a defective gene in conditions like cystic fibrosis.

Targeted Gene Modifications:

- Enables precise alterations to DNA to correct errors or enhance traits.
- Example: Correcting point mutations in sickle cell anaemia.





2. Applications in Agriculture

CRISPR-Cas9 is used to improve crop productivity, resistance, and nutritional value:

Improving Productivity:

- Modifications enhance growth, photosynthesis efficiency, or drought resistance.
- ☑ Example: Engineering crops to survive arid climate.

Enhancing Disease Resistance:

- ✓ Targeted edits make crops resistant to diseases or pests.
- ☑ Example: Bananas engineered to resist Panama disease.

Increasing Nutritional Value:

- Edits improve the nutritional profile of crops.
- ☑ Example: Golden rice enriched with Vitamin A.

3. Genetically Modified Organisms (GMOs) and Transgenic Organisms

CRISPR-Cas9 plays a critical role in creating GMOs for agricultural and research purposes:

Genetically Modified Organisms (GMOs):

- Organisms whose genomes have been altered artificially.
- ✓ Includes any form of genetic modification, whether involving foreign or native genes.

Transgenic Organisms:

- ✓ A subset of GMOs, where genes from an unrelated species are introduced.
- Example: Adding genes from fish to tomatoes for cold resistance.

Cisgenic Organisms:

- ✓ Involves transferring genes from the same or closely related species.
- Example: Enhancing disease resistance in crops using native plant genes.



Section E: Bioethics in VCE



How do you know what the "right" thing to do is?

Definition

What Knowledge is Required?

- Scientists, particularly in a field such as Biology, which is intrinsically related to humans ourselves, need to have some understanding of the ethical implications of their behaviours and their experiments.
 - Prevents abuses being made- animal abuse, discrimination (think WW2 experiments) and other human rights violations that might occur. Not only this, but some of the intellectual implications (plagiarism, reporting false data) also need to be considered.
- We have approaches and principles that help us determine the ethical nature of our actions.

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Sub-Section: Approaches to Bioethics



Approaches to Bioethics



- There are three major approaches to resolving ethical issues that support students in identifying bioethical issues, exploring these bioethical issues in context, considering different perspectives on bioethical issues, reflecting on courses of action, and choosing a position or course of action on the basis of reasoning and reflection.
- ➤ The Consequences-based approach places central importance on the consideration of the consequences of an action (the ends), with the aim of achieving the maximisation of positive outcomes and minimisation of negative effects.
- The Duty- and/or 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.
- The Virtues-based approach is person-based 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 so that they can act in the right way.

Discussion: Applying a Consequentialist Approach....



- Situation: A new vaccine is developed that is effective against a deadly virus, but has rare severe side effects.
- **Ethical Dilemma**: Should mass vaccination programs be initiated despite the potential for rare, but serious harm to individuals?
- Consequentialist Analysis:

Consequentialist Analysis: The decision to proceed with mass vaccination would be based on a utilitarian calculation. If the vaccine is likely to save millions of lives while only a small number might suffer from side effects, the action that maximizes overall well-being (mass vaccination) is considered the ethically correct one.



Discussion: Applying a Duty-Based Approach...



- **Situation**: A doctor has a patient who could benefit from a new experimental treatment, but the treatment has not been fully approved by ethical review boards.
- Ethical Dilemma: Should the doctor administer the treatment to help the patient, potentially disregarding the established approval processes?
- Deontological Analysis:

Deontological Analysis: Even if the treatment could save the patient's life, a deontologist would argue against its use until it has passed all required ethical approvals. The doctor has a duty to follow the medical ethical principle of "do no harm," which includes adherence to established medical standards and regulations.

Discussion: Plagiarism



- Situation: A nurse dealing with a terminal patient who is in severe pain and has expressed a desire to stop all treatment.
- Ethical Dilemma: Should the nurse advocate for the cessation of treatment, respecting the patient's wishes, or continue treatment as advised by the healthcare team?
- Virtue Ethics Analysis:

Virtue Ethics Analysis: A virtues-based approach would have the nurse consider what a compassionate and courageous healthcare professional would do. Advocating for the patient's wishes to be respected might be seen as embodying the virtue of compassion, while also having the courage to challenge the healthcare team if necessary to honor the patient's autonomy and dignity.

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Sub-Section: Bioethical Concepts



Ethical Concepts



While there are many ethical concepts that can support the investigation of bioethical issues, one or more of the following principles should be applied:

Integrity



- The commitment to searching for knowledge and understanding and the honest reporting of all sources of information and communication of results, whether favourable or unfavourable, in ways that permit scrutiny and contribute to public knowledge and understanding.
 - Not faking your results.
- Honesty is the best policy, have good intentions.







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- The moral obligation to ensure that there is fair consideration of competing claims, that there is no unfair burden on a particular group from an action, and that there is fair distribution and access to the benefits of an action.
- Ensuring that there is equity in access to things ensuring that all participants in an experiment are treated fairly.



Beneficence



- The commitment to maximising benefits and minimising the risks and harms involved in taking a particular position or course of action.
 - **G** E.g. Helping an old lady cross the street and pick up her groceries.
- Do good things.



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Non-maleficence



- Involves avoiding the causes of harm. However, as positions or courses of action in scientific research may involve some degree of harm, the concept of non-maleficence implies that the harm resulting from any position or course of action should not be disproportionate to the benefits from any position or course of action.
 - E.g. Cutting someone open for surgery (harm) to save their life (good).
- Don't do bad things, unless you have to for a good outcome.



Respect



- Involves consideration of the extent to which living things have an intrinsic value and/or instrumental value; giving due regard to the welfare, liberty and autonomy, beliefs, perceptions, customs and cultural heritage of both the individual and the collective; consideration of the capacity of living things to make their own decisions; and, when living things have diminished capacity to make their own decisions, ensuring that they are empowered where possible and protected as necessary.
 - Respecting that people have a right to their beliefs and make their own decisions.
 - Respecting the value of life and people's dignity.
 - E.g.- Not using the data of a routine survey if they did not consent to this data being used in a research study.

TIP: VCAA does not want you to be fully sound in your ethical reasoning like philosophers would - however, you need to have a basic understanding of these concepts so you can apply them to unique situations!





Sub-Section: General Ethical Scenarios



<u>Overview</u>



- To begin with, these scenarios are not made to be indicative of the types of questions you might face, rather, they are here to help us understand these concepts before moving on to an explanation and application.
 - For each of these, consider whether the nature of the action taken was ethical, using the approaches, and consider both the positives and negatives of the case, based on the principles we have learned as well.

Discussion: The Coronavirus Pandemic



- Italian hospitals were overrun during the pandemic, especially during the earlier first waves. How could the doctors decide which patients to treat over others?
- There is no right answer but work through it using ethical approaches.
- Consequences?
- Duty-Based?
- Virtue-Based?

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A consequences-based approach might argue that each hospital should aim to maximise good for the greatest number of people. If it turned out, as was the case for most Italian hospitals, that the demand for care outweighed their resources, then maximising good outcomes might mean focusing those resources on patients who are likely to best recover and respond to treatment. In this case, that means focusing treatment on young and otherwise healthy individuals, and less so on older patients who are perhaps less likely to survive.

A duty/rule-based approach might argue that as doctors and healthcare providers, each individual has a moral obligation to provide treatment to whoever is in need. Perhaps, this would mean focusing resources in a first-come-firstserve manner, where care is distributed sequentially based on when a patient arrives at the hospital. A duty/rule-based approach might suggest that this is the fairest means of distributing care and that hospitals have a duty to act indiscriminately rather than turning away patients based on their age.

A virtues-based approach might argue that doctors ought to follow the moral guidance of their own value judgements as to who is most in need. That is, the distribution of care should be left to the moral virtues of a good doctor - such as kindness, fairness and good judgment. In this case, a hospital would not follow a certain rule (such as first-come-first-serve), nor focus on maximising consequences (though this may well be an outcome). Instead, each doctor is seen as their own moral agent deciding how best to distribute resources.

Discussion: Plagiarism



Which ethical principle/s would plagiarism most likely be in breach of?



<u>Discussion:</u> Running Clinical Trials for New Medications



Which ethical principle/s would we have to consider?

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Sub-Section: Applying Ethics to GMOs



Implications of GMOs



- We need to consider the biological, social and ethical implications of this, looking at both the positives and the negatives.
- Biological?
 - Better productivity so that they take up less and, therefore, there are reduced land clearing rates.
 - Insect and disease resistance means that fewer chemicals are needed.
 - Fewer crop losses due to the resistance.
 - Improved nutrition.
 - Lose effectiveness if weeds and diseases evolved.
 - Reduction of genetic diversity if populations are derived from a few edited organisms.
 - Cross-pollination may inadvertently cause these GM crops to be released into the wild, causing genes to spread unforeseen consequences.
- Social?
 - Better food security.
 - Protect against famine.
 - Farmers have to spend less effort and money protecting their plants.
 - Better produce due to the engineering.
 - Companies may control the genetically modified seed giving them power over farmers.
 - Legal issues may cause farmers problems.
 - People may be uninformed of the presence of GMOs and make a decision.



Ethical? Think about the ethical frameworks we have discussed earlier.

Cons

- Having to buy new seeds each season may be costly for farmers.
- Complex legal issues surrounding the use of GM products may cause farmers undue stress and anxiety related to regulation.
- There are strict packaging and marketing regulations for GMO producers that may not be complied with, if either the producer or consumer are undereducated on these regulations.

Pros

Some people believe that using genetic modification is an ethical imperative given the potential for widespread benefits, including nutrition, wealth and the overall health of humanity, especially in developing nations.

Cons

- Some people consider GMOs to be unnatural or like we are 'playing God'.
- Some people believe that GM foods are unsafe to eat and choose not to eat them as a result. This is especially true if there is a lack of long-term evidence of healthy use.
- Some people believe that genetically modifying animals for human benefit is inhumane many anti-animal GMO arguments apply to animal agriculture in general.
- The fact that companies can own the rights to GM crops is considered by some to be unethical due to companies possibly making unfair demands from farmers. This ownership power divide can materialise in a range of ways, including the following:
 - Cross-pollination of non-GM crops by nearby GM crops could result in the non-GM farmer being sued by the patent-owner.
 - Farmers can't reuse seeds from some GM crops and must buy new expensive seed supplies each year from biotechnology companies.

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Sub-Section: Applying Ethics to CRISPR-Cas9



Applying Ethics to CRISPR-Cas9



- Considering the bioethical principles that we have studied, how could they be applied to CRISPR?
 - Beneficence?

We have an obligation to help - if CRISPR can help, then why not?

Non-maleficence?

Do no harm - there are significant concerns about the safety of the technology at this given point in time. There is unforeseen impact that could implicate the edited embryo or organism to trauma later along the line.

Consent?

The embryo cannot consent to edits and may be subject to changes to its phenotype which may be not be consented to. Also, it has to be INFORMED that the patients must fully know what is going on and that includes the parents, especially about the potential impacts of the technology.

Justice?

Inequality and discrimination faced by those who has edits compared to those who cannot, the prospect of people just editing for the loss, the financial disadvantage might entrench further inequality.



<u>Discussion:</u> Let's talk about the Chinese scientist who did gene editing illegally!



What was wrong ethically with that? Refer to the approaches that we have learned as well as the principles.

https://www.nature.com/articles/d41586-018-07545-0

Head Tutor's Note: Search up He Jiankui on Youtube to show his actual interview !!!

Key Takeaways

1. Ethical Approaches

- Ethical approaches provide frameworks for analysing and resolving ethical dilemmas, particularly in the context of CRISPR-Cas9 and other biotechnologies:
 - Consequences-Based Approach:
 - Central focus: Outcomes or consequences of an action.
 - ☑ Goal: Maximise positive effects and minimise harm.
 - Example: Supporting CRISPR use for disease eradication if the societal benefits outweigh risks.
 - Critique: Can justify morally questionable actions if the outcomes are favourable.
 - Outy/Rule-Based Approach:
 - Central focus: Adherence to moral rules or duties, regardless of outcomes.
 - Emphasis on ethical responsibilities and strict regulations.
 - Example: Requiring full regulatory approval before using CRISPR on embryos.
 - Critique: May ignore potential benefits if rules are overly restrictive.



Virtues-Based Approach:

- ☑ Central focus: The moral character and intentions of the individual performing the action.
- M Emphasis on qualities such as honesty, fairness and humility.
- Example: Scientists exercising restraint and prioritising ethical considerations in CRISPR applications.
- Critique: Relies on subjective interpretations of virtue.

2. Ethical Concepts

☑ These principles guide ethical decision-making by providing a moral foundation for evaluating actions:

Integrity:

- ☑ Definition: A commitment to honesty and accurate reporting.
- ☑ Ensures transparency and trust in scientific research.
- ☑ Example: Publishing both positive and negative results from CRISPR experiments.

Respect:

- ☑ Definition: Recognising the intrinsic value and autonomy of living things.
- ✓ Considers welfare, cultural beliefs and personal rights.
- ☑ Example: Gaining informed consent before experimenting on human subjects.

Beneficence:

- ☑ Definition: Maximising benefits while minimising risks and harms.
- Encourages actions that contribute positively to individuals or society.
- ☑ Example: Using CRISPR to cure genetic disorders responsibly.

Non-Maleficence:

- Definition: Avoiding harm or ensuring harm is proportionate to the benefits.
- Balances risk and reward in scientific practices.
- Example: Carefully evaluating potential off-target effects of CRISPR edits.



Justice:

- ☑ Definition: Ensuring fair treatment, distribution of benefits and avoidance of undue burdens.
- Focus on equity and accessibility.
- ☑ Example: Avoiding socio-economic inequalities in access to CRISPR-based therapies.

Summary of the Implications of GMOs

☑ Biological Implications:

Positives:

- Enhanced productivity reduces land use and deforestation.
- ✓ Increased insect and disease resistance decreases chemical use.
- ✓ Fewer crop losses due to better resistance mechanisms.
- ✓ Improved nutrition through genetic enhancements (e.g., fortified crops).

• Negatives:

- ☑ Evolution of resistant weeds and diseases may reduce effectiveness.
- Reduction in genetic diversity if populations rely on a few modified strains.
- Cross-pollination may spread modified genes to wild species, causing unforeseen ecological consequences.

✓ Social Implications:

Positives:

- ✓ Improved food security and famine protection.
- ☑ Reduced costs and effort for farmers in managing crops.
- Higher quality produce due to genetic engineering.

Megatives:

- Corporate control over GM seeds may disadvantage farmers.
- Legal issues and regulations can burden farmers.
- Lack of public awareness about GMOs may lead to uninformed decisions.



✓ Ethical Implications:

Cons:

- Farmers face recurring costs for new seeds annually.
- ☑ Strict regulations and packaging requirements create challenges for compliance.
- ☑ Concerns about GMOs being "unnatural" or interfering with nature ("playing God").

Pros:

Many consider genetic modification ethically imperative for addressing malnutrition, poverty, and health issues, particularly in developing countries.

Summary of the Ethical Applications of CRISPR-Cas9

Beneficence:

Obligation to use CRISPR to maximise benefits, such as curing genetic diseases and improving crop productivity.

Non-Maleficence:

Avoid causing harm, considering unknown long-term effects or off-target mutations that could negatively impact health or the environment.

Consent:

☑ Ethical concerns arise in germline editing where future generations cannot provide consent. Ensuring parents and patients are fully informed is critical.

Justice:

CRISPR's potential to widen socio-economic inequalities, with only wealthy individuals having access to enhancements, must be addressed.







Contour Check

<u>Learning Objective</u>: [1.6.1] - Describe the Function & Process of CRISPR-Cas9 as an Adaptive Defence Against Viruses in Bacteria

Study Design

The function of CRISPR-Cas9 in bacteria and the application of this function in editing an organism's genome.

Key Takeaways

- CRISPR-Cas9 is a revolutionary tool derived from bacteria's natural defence mechanism against viruses (bacteriophages).
 - O It stands for Clustered Regularly Interspaced Short Palindromic Repeats.
 - O Function: Allows for precise and efficient gene editing by targeting specific DNA sequences.
- Analogy:

Overview:

- O It works like a surveillance system: bacteria "store" viral DNA to recognise and neutralise future invasions.
- Key Features:
 - Cas9: A programmable enzyme (endonuclease) that cuts DNA.
 - Guide RNA (gRNA): Guides Cas9 to the specific target DNA via complementary base pairing.
 - PAM (Protospacer Adjacent Motif): A short DNA sequence that enables Cas9 to bind and cut at the correct site.
- Process:
 - Exposure: A bacteriophage injects its DNA into a bacterial cell.
 - Endonucleases cut a protospacer (a small viral DNA fragment) adjacent to a PAM sequence.



- Incorporation: The protospacer is integrated into the bacterial genome.
- Expression: The spacer is transcribed into crRNA and combines with tracrRNA to form guide RNA (gRNA).
- Extermination: gRNA directs Cas9 to recognise and cut complementary viral DNA, inactivating the virus.

<u>Learning Objective</u>: [1.6.2] - Explain the Process of Using CRISPR-Cas9 as a Gene Editing Tool, Including Silencing, Knock-Ins & Knock-Outs

Study Design

The function of CRISPR-Cas9 in bacteria and the application of this function in editing an organism's genome.

Key Takeaways

Overview:

- 1. CRISPR-Cas9 has been adapted as a powerful and versatile tool for editing genomes in eukaryotic cells.
- 2. The system uses the principles of bacterial immunity but has been optimised to make precise cuts in specific DNA sequences within any organism.

■ How it Works:

- 1. Target Sequence Identification:
 - A target DNA sequence is selected for editing, such as a gene responsible for a disease.
 - Researchers design synthetic **single guide RNA (sgRNA)**, which combines the targeting properties of crRNA and tracrRNA into one molecule.
- 2. CRISPR-Cas9 Complex Formation:
 - sgRNA binds to the Cas9 enzyme, forming the CRISPR-Cas9 complex.



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- ☐ The complex locates the target DNA by matching the sgRNA sequence to the complementary DNA sequence.
- A **PAM sequence** (e.g., NGG) adjacent to the target ensures proper binding and initiates the cutting process.

4. DNA Cutting:

Cas9 creates a double-strand break at the target site.

5. Repair Mechanisms:

- The cell's natural repair mechanisms are activated:
 - Non-Homologous End Joining (NHEJ): Repairs the break but often introduces errors (e.g., insertions or deletions), which can disable the gene (gene knock-out).
 - Homology-Directed Repair (HDR): Uses a provided DNA template to repair the break precisely, allowing the insertion of new genetic material (gene knock-in).

6. Gene Silencing (Knock-Outs):

- Cas9 introduces a double-strand break at a target DNA sequence.
- The cell repairs the break via non-homologous end joining (NHEJ), which often introduces mutations, disabling the gene.
- Example: Disabling genes linked to diseases like cancer or Huntington's disease.

7. Gene Knock-Ins:

- Homology-directed repair (HDR) uses an introduced template to insert new DNA sequences.
- Example: Adding a functional copy of a defective gene in conditions like cystic fibrosis.

8. Targeted Gene Modifications:

- Enables precise alterations to DNA to correct errors or enhance traits.
- Example: Correcting point mutations in sickle cell anaemia.



<u>Learning Objective</u>: [1.6.3] – Describe & Compare the Function of the PAM Sequence in Bacteria & Gene Editing Applications of CRISPR-Cas9 Technology

Study Design

The function of CRISPR-Cas9 in bacteria and the application of this function in editing an organism's genome.

Key Takeaways

- ☐ Role of PAM:
 - PAM ensures that Cas9 binds to and cuts only the foreign DNA, distinguishing self from nonself-DNA. It also ensures that the Cas9 will only bind to DNA and unwind it for detecting a match where it is possible.
 - This system is highly specific and efficient, making it adaptable for gene editing.
 - O In gene editing applications, it allows the system to be more precise and efficient.

<u>Learning Objective</u>: [1.6.4] - Describe the Function & Compare the Guide RNA (gRNA) & Single Guide RNA (sgRNA)

Study Design

The function of CRISPR-Cas9 in bacteria and the application of this function in editing an organism's genome.

Key Takeaways

- Guide RNA (gRNA) is a combination of crRNA (CRISPR RNA) and tracrRNA (trans-activating crRNA), which directs Cas9 to the target DNA by complementary base pairing.
- Single guide RNA (sgRNA) is a synthetic RNA that combines the functions of crRNA and tracrRNA into one molecule for simplicity and efficiency.



■ Both gRNA and sgRNA guide Cas9 to specific DNA sequences for cutting.		
The sgRNA simplifies the CRISPR-Cas9 system by eliminating the need for two separate RNA molecules.		
■ Both types are critical for ensuring precise targeting in genome editing applications.		
<u>Learning Objective</u> : [1.6.5] - Apply Bioethical Principles to the Use of CRISPR- Cas9 Technology		
Study Design		
The use of genetically modified & transgenic organisms in agriculture to increase crop productivity & to provide resistance to disease.		
Key Takeaways		
□ Beneficence:		
 Obligation to use CRISPR to maximise benefits, such as curing genetic diseases and improving crop productivity. 		
□ Non-Maleficence:		
 Avoid causing harm, considering unknown long-term effects or off-target mutations that could negatively impact health or the environment. 		
Consent:		
 Ethical concerns arise in germline editing where future generations cannot provide consent. Ensuring parents and patients are fully informed is critical. 		
□ Justice:		
 CRISPR's potential to widen socio-economic inequalities, with only wealthy individuals having access to enhancements, must be addressed. 		



<u>Learning Objective</u>: [1.6.6] - Define & Describe the Bioethical Concepts of Integrity, Respect, Beneficence, Non-Maleficence & Justice as Elaborated in the VCAA Study Design

Study Design

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Key Takeaways

These principles guide ethical decision-making by providing a moral foundation for evaluating actions:

- Integrity:
 - Definition: A commitment to honesty and accurate reporting.
 - Ensures transparency and trust in scientific research.
 - Example: Publishing both positive and negative results from CRISPR experiments.
- Respect:
 - O Definition: Recognising the intrinsic value and autonomy of living things.
 - Considers welfare, cultural beliefs and personal rights.
 - Example: Gaining informed consent before experimenting on human subjects.
- Beneficence:
 - O Definition: Maximising benefits while minimising risks and harms.
 - Encourages actions that contribute positively to individuals or society.
 - Example: Using CRISPR to cure genetic disorders responsibly.



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- O Definition: Avoiding harm or ensuring harm is proportionate to the benefits.
- O Balances risk and reward in scientific practices.
- Example: Carefully evaluating potential off-target effects of CRISPR edits.

Justice:

- O Definition: Ensuring fair treatment, distribution of benefits and avoidance of undue burdens.
- Focus on equity and accessibility.
- Example: Avoiding socio-economic inequalities in access to CRISPR-based therapies.



<u>Learning Objective</u>: [1.6.7] - Define & Describe the Three Ethical Approaches as Elaborated in the VCAA Study Design

Study Design

Pg 15-16

Key Takeaways

- Consequences-Based Approach:
 - Central focus: Outcomes or consequences of an action.
 - Goal: Maximise positive effects and minimise harm.
 - Example: Supporting CRISPR use for disease eradication if the societal benefits outweigh risks
 - Critique: Can justify morally questionable actions if the outcomes are favourable.
- Duty-/Rule-Based Approach:
 - Central focus: Adherence to moral rules or duties, regardless of outcomes.
 - Emphasis on ethical responsibilities and strict regulations.
 - Example: Requiring full regulatory approval before using CRISPR on embryos.
 - Critique: May ignore potential benefits if rules are overly restrictive.
- ☐ Virtues-Based Approach:
 - Central focus: The moral character and intentions of the individual performing the action.
 - Emphasis on qualities such as honesty, fairness and humility.
 - Example: Scientists exercising restraint and prioritising ethical considerations in CRISPR applications.
 - Critique: Relies on subjective interpretations of virtue.



<u>Learning Objective</u>: [1.6.8] - Describe Briefly How to Genetically Modify Organisms to Increase Crop Productivity & Disease Resistance, Using CRISPR-Cas9

Study Design

The use of genetically modified & transgenic organisms in agriculture to increase crop productivity & to provide resistance to disease.

Key Takeaways

CRISPR-Cas9 is used to improve crop productivity, resistance and nutritional value:

- Improving Productivity:
 - O Modifications enhance growth, photosynthesis efficiency or drought resistance.
 - O Example: Engineering crops to survive arid climates.
- Enhancing Disease Resistance:
 - Targeted edits make crops resistant to diseases or pests.
 - Example: Bananas engineered to resist Panama disease.
- Increasing Nutritional Value:
 - O Edits improve the nutritional profile of crops.
 - Example: Golden rice enriched with Vitamin A.



<u>Learning Objective</u>: [1.6.9] - Compare Transgenic, Cisgenic & Genetically Modified Organisms

Study Design

The use of genetically modified & transgenic organisms in agriculture to increase crop productivity & to provide resistance to disease.

Key Takeaways

CRISPR-Cas9 plays a critical role in creating GMOs for agricultural and research purposes:

- Genetically Modified Organisms (GMOs):
 - Organisms whose genomes have been altered artificially.
 - O Includes any form of genetic modification, whether involving foreign or native genes.
- Transgenic Organisms:
 - A subset of GMOs where genes from an unrelated species are introduced.
 - Example: Adding genes from fish to tomatoes for cold resistance.
- Cisgenic Organisms:
 - Involves transferring genes from the same or closely related species.
 - Example: Enhancing disease resistance in crops using native plant genes.



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