How Ethical is CRISPR-Cas9 and What is it?

Eva Elena Mannsbart
Department of Biology
Lake Forest College
Lake Forest, Illinois 60045

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Artificial intelligence was used during the writing of this essay. In the "Applications" section, AI helped brainstorm examples of CRISPR applications, including treatments for leukemia, improving crop yields, and the modification of malaria-carrying mosquitoes such as *Anopheles gambiae*. Moreover, AI helped summarize my 3 primary research papers. In the "Ethics" section, AI suggested I first discuss the ethics of the research papers and then expand to broader issues. This guidance improved the flow and clarity of my ideas. AI also assisted with finding credible sources and formatting citations. Overall, artificial intelligence was used to help organize, generate ideas, and strengthen the academic quality of this essay.

Introduction

Imagine waking up one day to discover that an incurable disease you have mysteriously vanished. This is exactly what happened to a woman with a rare hereditary disorder called WHIM syndrome (Doudna, 2017). Researchers studying the disease were astounded when the illness miraculously disappeared. It seems that a single stem cell underwent a spontaneous change that rid the cell of the disease. What happened was essentially a naturally occurring gene edit: her body had genetically modified its DNA, eliminating the disease (Doudna, 2017). Such a spontaneous cure is extraordinarily rare. Natural gene editing like this remains a medical anomaly. But what if gene editing no longer had to rely on chance?

Gene editing is something scientists have long aspired to achieve. With the discovery of a revolutionary technology called CRISPR, they are now closer than ever. CRISPR-Cas9 stands for *Clustered Regularly Interspaced Short Palindromic Repeats*. While the full name is technical and lengthy, the acronym refers to a powerful tool that enables precise gene editing. Interestingly, the CRISPR mechanism was not invented by humans; it was adapted from bacteria. Scientists observed that bacteria used CRISPR as a defense mechanism against viruses and later repurposed it for gene editing in other organisms.

This paper will explore the CRISPR mechanism in detail, beginning with its biological origins and its adaptation for gene editing. It will then examine a specific real-world application: the use of CRISPR to genetically modify malaria-transmitting mosquitoes. Finally, the paper will examine the ethical dilemmas that inevitably arise from gene-editing technologies. Overall, this paper will analyze CRISPR from three key perspectives: scientific, examining its mechanism; practical, through its real-life applications; and ethical, through a discussion of the controversies it raises.

CRISPR Mechanism

Often, when creating new things, humans draw inspiration from nature. For example, the design of Velcro was inspired by burrs and their ability to stick to animal fur with their small hooks (Science Reference Section, 2019). Similarly, an aspect of gene editing was inspired by a naturally occurring immune response in bacteria. Bacteria are prokaryotes whose greatest threat comes from viruses known as bacteriophages. To defend themselves against viral attacks, bacteria have developed a fascinating immune system called CRISPR. Just as burrs inspired the development of Velcro, this system of bacterial immunity inspired an aspect of gene editing. To understand the connection between the bacterial immune system and gene editing, it is first necessary to examine how the bacterial immune system functions.

Bacterial immunity against viruses was researched in the Danisco study (Doudna, 2017). In the dairy industry, products like cheese are made using *Streptococcus thermophilus*, a bacterium that ferments milk. Danisco, a major dairy company, noticed that its production was suffering because large numbers of its milk-fermenting bacteria were being killed by bacteriophages. To address this problem, they funded a study to understand how some bacteria seem to survive viral attacks. In the study, scientists mixed *S. thermophilus* with bacteriophages and found that while 99.9% of the bacteria died, a small fraction of mutant strains survived. After isolating genomic DNA from each mutant strain, the researchers found that all had a DNA sequence matching that of the bacteriophage. These matching copies conferred immunity on the bacteria. Moreover, this immunity, since it is stored in the bacteria's DNA, is heritable. This discovery meant the Dancisco's company could increase its production yields by selecting bacterial strains resistant to bacteriophages (Doudna, 2017).

Now that scientists understood why some bacteria were resistant to bacteriophages, they aimed to determine the precise mechanisms responsible for this immunity. As previously mentioned, scientists realized that the surviving bacterium shared some DNA with the virus. This is because when a bacterium survives a viral attack, it copies some viral DNA and stores it in a specific area of its genome, called the CRISPR array. This region contains a sequence that alternates between repeated bacterial DNA and foreign viral DNA. Essentially, it works as a filing system, keeping track of all the virus infections that the bacteria have survived. If the same virus tries to attack again later, the bacterium can defend itself. It does this by first creating a guide RNA (gRNA) from the stored viral DNA. As its name suggests, this guide RNA directs the Cas9 protein to the matching sequence in the invading viral DNA. Once Cas9 binds to the target viral DNA, it makes a double-stranded cut, destroying the virus before it can cause harm. This system acts like an immune memory, allowing bacteria to quickly recognize and fight off repeat infections (Prillaman, 2024). Therefore, scientists often describe this process of recognition as "a molecular vaccination card" (Doudna, 2017).

The Cas9 protein, which cuts viral DNA, is the key link between the bacterial immune system and modern gene editing. Scientists such as Jennifer Doudna were struck by Cas9's ability to recognize specific viral DNA sequences. They hypothesized that this natural precision could be repurposed to target almost any chosen DNA sequence. The first step in this process is designing the appropriate gRNA, which guides Cas9 to the target DNA. To do this, the gRNA must match the target DNA sequence, typically with a sequence of about 20 nucleotides (Thurtle-Schmidt & Lo, 2018). This level of specificity is significant because, while short DNA sequences are often repeated throughout the genome, a 20-nucleotide match reduces the risk of targeting the wrong site. However, even a perfect match between the gRNA and target DNA is not sufficient on its own. Cas9 will only cut the DNA if a short sequence known as PAM (Protospacer Adjacent Motif) is located immediately next to the target site, as illustrated in *Figure 1*. Cas9 can therefore be used to both locate and cut any desired gene.

Having established Cas9's ability to precisely locate and cleave specific genes, the next consideration is how gene editing is carried out. Sickle cell anemia provides a clear example, as it results from a single-base mutation in the HBB gene, in which glutamic acid is replaced by valine (Pattabhi et al., 2019). CRISPR can correct this mutation by using a guide RNA (gRNA) to direct Cas9 to the precise location of the faulty sequence. Cas9 then creates a double-strand break in the DNA. To repair this break, the cell uses a process called Homology-Directed Repair (HDR). A donor DNA template containing the correct gene without the sickle cell mutation is provided alongside Cas9. The cell then uses this template to accurately copy the correct DNA sequence. Through HDR, the mutated segment is replaced with the normal sequence, effectively editing the HBB gene (Pattabhi et al., 2019).

The Cas9 complex used by bacteria has been modified to increase its practicality in gene editing. In the natural CRISPR-Cas9, two types of RNA make up the gRNA: CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA). The crRNA contains a sequence complementary to the target viral DNA, enabling precise recognition. Meanwhile, the

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tracrRNA binds to the crRNA, helping form a stable Cas9 complex. To facilitate gene editing, scientists created a chimeric form of these two RNAs, which they called a single guide RNA (sgRNA) or a chimeric RNA. The chimeric RNA was tested to determine whether it could still function in guiding Cas9 to the target DNA. The results of this experiment are shown in the gel electrophoresis of *Figure 2*. This technique separates DNA fragments by size. If the DNA is cut by Cas9, multiple fragments will appear along a band. 5 versions of the chimeric RNA were tested, as labeled on the x-axis. In lanes containing chimeric RNA, multiple DNA fragments are visible. This indicates that the Cas9 was successfully directed to the target sequence (Jinek et al., 2012). Therefore, scientists were able to significantly facilitate the process of gene editing.

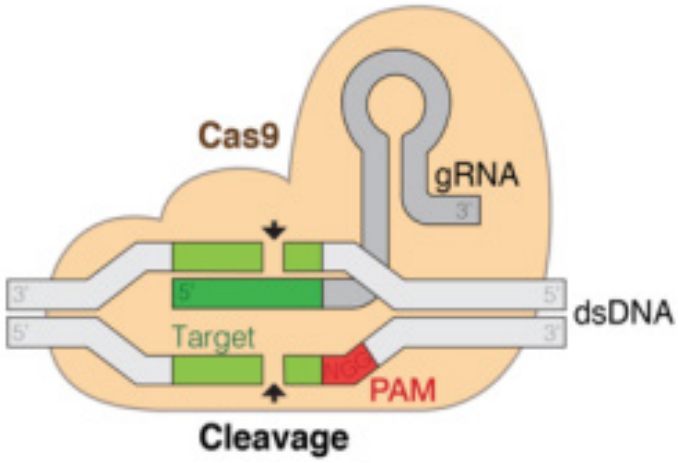

Figure 1: Cas9 protein complex (Lohner, 2021).

Applications

CRISPR technology has been used to genetically modify a wide range of organisms. This paper will examine how CRISPR is used to genetically modify *Anopheles gambiae*, the mosquito species that transmits malaria. Malaria, which kills over 600,000 people annually, is caused by the *Plasmodium* parasite, a type of single-celled eukaryote (WHO, 2024). The parasite enters mosquitoes, and when female mosquitoes feed on human blood, their saliva transmits the parasite into the human bloodstream. The parasite then travels to the liver, where it grows and matures. Once back in the bloodstream, it infects red blood cells, multiplying within them until the cells rupture, releasing even more parasites. This cycle continues, often leading to death. To combat this, scientists are exploring two CRISPR-based techniques. One involves reducing mosquito populations by inducing infertility, and the other focuses on preventing the parasite from infecting mosquitoes in the first place. The following sections will explore how these methods work to decrease the spread of malaria.

CRISPR technology is used to spread infertility in female *A. gambiae* mosquitoes, reducing their population and the spread of malaria (Nolan et al., 2016). A study by Andrea Crisanti and Tony Nolan et al. used CRISPR to insert a gene that causes infertility in female mosquitoes. To increase the chance that this infertility is inherited beyond the usual 50%, this study used a CRISPR-Cas9 gene drive. Once the desired gene is introduced to one chromosome, the gene drive copies it onto the unmodified one. This means that both the paternal and maternal chromosomes carry the gene, ensuring it is passed on to future generations. Since the gene causes infertility in females, it is the males who spread the infertility gene. Over time, as more females become infertile, the mosquito population significantly declines. The study showed that the gene drive was initially effective. However, it was also found that by the 2nd generation, the variants had become resistant to the CRISPR gene drive. This is a significant limitation, as it blocks the gene drive from being inherited, defeating its purpose of blocking the spread of malaria (Nolan et al., 2016).

To overcome resistance to gene drives, the same researchers utilized a different gene: the *doublesex* (dsx) gene (Nolan et al., 2018). This gene is crucial for female development and fertility. The scientist hypothesized that if a mutation in the gene drive with this gene occurred, it would cause infertility. CRISPR gene drives work by cutting the non-modified chromosome and copying the gene drive sequence onto it. To repair this cut, the cell often uses a process called non-homologous end joining (NHEJ), which is error-prone and can introduce small nucleotide insertions or deletions. It is precisely these seemingly small errors that lead to CRISPR resistance. In this study, although mutations would inhibit the gene drive, they would also cause the *doublesex* gene to cause infertility. In this strategy, even if resistance develops and the CRISPR gene drive fails, the female remains infertile. Thus, by incorporating the *doublesex* gene into the gene drive, the researchers created a foolproof system where infertility is inevitable, leading to the eventual collapse of the mosquito population (Nolan et al., 2018).

Introducing sterile insects is not the only way to reduce the spread of malaria; preventing mosquitoes from becoming infected in the first place is another strategy (Dimopoulos, 2018). In this approach, CRISPR is used to make *Anopheles gambiae* mosquitoes less susceptible to *Plasmodium* infection. For the parasite to develop in the mosquito, it requires numerous host factors, called agonists. If agonists are removed, the parasite cannot develop or infect the mosquito. In a study by George Dimopoulos et al., CRISPR is used to knock out the *FREP1* gene, an important agonist. The results showed that mosquitoes lacking the *FREP1* gene were less susceptible to *Plasmodium* infection. Meaning they were not infected by the malaria-causing parasite. However, the gene knockout also caused significant fitness costs, including reduced blood-feeding ability and lower egg hatching rates. These fitness disadvantages suggest that genetically modified mosquitoes may be less competitive in the wild. This reduces their chances of surviving, reproducing, and passing down their increased resistance to parasites (Dimopoulos, 2018).

All three papers shared important similarities: they each used CRISPR to reduce the spread of malaria and employed similar experimental techniques. One key method microinjection to deliver the CRISPR components into the *A. Gambiae* embryos. This process begins by creating the desired Cas9 complex. In each study, the single-guide RNA (sgRNA) was designed with a base sequence complementary to the specific target gene. The microinjection mixes typically included not only the CRISPR-Cas9 construct but also fluorescent marker proteins to help identify successful gene editing. The fluorescent mosquitoes with edited genes were then crossed with wild-type mosquitoes. This process, known as backcrossing, helps maintain phenotypic variability and overall fitness, and serves as a form of selective breeding. All three studies shared significant similarities, the most important being their common goal of reducing the spread of malaria.

Ethics of CRISPR

The genetic modification of *A. gambiae* inevitably raises profound ethical concerns. Spreading infertility throughout a species, potentially leading to its extinction, must be carefully weighed. On one hand, disrupting the mosquito population could have ecological consequences that may affect other species. On the other hand, malaria is responsible for over 600,000 deaths annually (WHO, 2024). While driving *A. gambiae* to extinction may have risks, humans "may consider it unethical not to use germline editing to alleviate human suffering" (Isaacson, 2021) (p.355). This raises the question: Should scientists be able to control the viability of another species if they pose a threat to human health?

Moreover, the ethics surrounding gene editing often depend on the perspective one takes. An environmental scientist might be more opposed to gene drives, given their understanding of the potential ecological consequences. In contrast, a biomedical scientist may be more inclined to support gene editing, focusing on the medical benefits of eliminating diseases like malaria. Policymakers face the challenge of balancing competing interests to satisfy both environmental concerns and public health goals. Additionally, perspectives vary across countries; nations where malaria poses a greater public health threat may be

more supportive of gene editing, while others may view it as too great a risk. These differing viewpoints contribute to intense debates, raising difficult questions about which perspectives should take precedence.

As we evaluate the ethical boundaries of gene editing, it is important to ask which traits should be edited. As previously mentioned, sickle cell disease is due to a genetic mutation that affects red blood cells, impairing their ability to transport oxygen efficiently. Individuals who inherit two copies of the sickle cell gene (homozygous) experience severe symptoms requiring treatments such as monthly transfusions. However, individuals with only one copy (heterozygous carriers) are largely asymptomatic. In both cases, nevertheless, the patient gains partial immunity to malaria (Mayo, 2025). Using CRISPR to correct the sickle cell mutation would alleviate symptoms, but it would also eliminate protection against malaria. While increasing malaria risk might be acceptable to relieve severe symptoms in homozygous individuals, removing malaria resistance from asymptomatic carriers raises more complex ethical questions. How do we choose which to prioritize? Is gene editing sickle cell anemia simply replacing one problem with another?

Another example that highlights the ethical dilemmas surrounding gene editing is deafness. Deafness can be a disadvantage in various scenarios, especially for families with limited resources. While society might label deafness as a disability, many "consider deafness to be part of who they are rather than something to be cured" (Isaacson, 2021) (p.334). Society's view of deafness as a disability has led some to argue that gene editing could be used to "fix" it. Thus, it is important to ask: "How do we distinguish between traits that are true disabilities and ones that are disabilities because society is not good at adapting to them?" (Isaacson, 2021) (p.336). Is it morally acceptable to use gene editing to change traits that cause no inherent harm, but are harmful due to society's perception of them?

As the examples above illustrate, gene editing often exists in an ethical gray zone, neither wholly right nor wrong. Given human nature, gene editing will inevitably occur, making the real question how society chooses to respond to these ambiguous cases. A recent example involves Dr. He Jiankui, who genetically modified the first human embryos, an experiment that led to his imprisonment (Normile, 2019). Although his achievement was scientifically groundbreaking, it was conducted prematurely and therefore unethically. In response, the scientific community issued a statement condemning his work, declaring that "the procedure was irresponsible and failed to conform with international norms" (National Academies of Sciences, 2018). As dilemmas surrounding gene editing continue to emerge, scientists must hold each other accountable for unethical research, regardless of how impressive the results may be.

Overall, the ethics surrounding gene editing are highly nuanced. In my opinion, ethical guidelines should be adjusted based on the location. While worldwide regulations may be important for ensuring consistency, they risk oversimplifying the complexity of this issue. As claimed by the Harvard Gazette, "It's very hard to deal with a transnational problem with national legislation" (Bergman, 2019). Instead, to address gene editing's ethical challenges effectively, each scenario should be evaluated on an individual basis. Moreover, it's important to "require students to learn the moral dimensions of science and technology", including that of gene editing. This will form much-needed public opinions (Bergman, 2019). Diverse perspectives, the cultural context of the countries, and the views of both the scientific community and the public must be considered in debates about gene editing.

Conclusion

Overall, it is evident that an extremely powerful tool for gene editing has emerged. This tool, CRISPR-Cas9, was inspired by an immune response found in bacteria. In nature, CRISPR enables bacteria to recognize specific viral DNA and destroy viruses before they can cause harm. Scientists were struck by Cas9's ability to recognize specific viral DNA and repurposed it to target almost any gene sequence. In gene editing, Cas9 is used to locate a specific gene and introduce a double-stranded break. The cell then repairs this break through either Homology-Directed Repair (HDR) or Non-Homologous End Joining (NHEJ), essentially editing the genome. Although this process exhibits off-target effects, minimizing them is a priority amongst researchers.

Considering the pace of technological advancements, the use of CRISPR in the future is inevitable. The question is no longer whether gene editing will occur but rather when and how (Doudna, 2017). The story of the woman cured of WHIM syndrome by chance is no longer just a medical marvel; it is a symbol of what science has made ordinary. Scientifically, CRISPR's repurposing was nothing short of revolutionary. But just as Doudna realized she could not explore CRISPR without also confronting its ethical implications, we too must recognize that scientific progress and ethical responsibility are inseparable. Gene editing holds incredible power, and therefore immense responsibility.

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