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Popular Article

CRISPR/Cas9 Gene Editing System in Livestock Animals

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Introduction

Genome editing is a technique used to make precise changes to the DNA sequence of an organism. It involves using molecular tools to add, delete, or replace specific genetic information. This technology has the potential to transform many areas of science and medicine, including agriculture, biotechnology, and animal health. There are several methods of genome editing, but the most widely used is the CRISPR/Cas9 system. This system is derived from a naturally occurring bacterial immune system and involves using a small RNA molecule to guide a protein called Cas9 to a specific location in the genome. Once at the target site, the Cas9 protein cuts the DNA, allowing researchers to add, delete, or replace specific genetic information. The discovery and development of CRISPR/Cas9 is widely considered one of the most important scientific breakthroughs of the 21st century. In 2020, Jennifer Doudna and Emmanuelle Charpentier were awarded the Nobel Prize in Chemistry for their work in developing the CRISPR/Cas9 gene-editing technology (Strzyz, 2020). Their research laid the foundation for a new era in biotechnology and has the potential to revolutionize the way we treat genetic diseases, develop new crops, and even create new materials. Doudna and Charpentier's work on CRISPR/Cas9 has been widely recognized by the scientific community, and their Nobel Prize is a testament to the significance of their discovery. It is also a testament to the potential of basic scientific research to have a profound impact on our lives and our

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world. Genome editing has many potential applications. In agriculture, it could be used to create crops that are more resistant to disease or have higher yields. In biotechnology, it could be used to create new therapies for genetic diseases. In basic research, it could be used to study the function of genes and their role in disease. However, genome editing also raises important ethical and social questions. For example, there are concerns about the potential unintended consequences of making changes to an organism's DNA, as well as the potential for creating new forms of inequality or discrimination. As a result, the use of genome editing is subject to careful scrutiny and regulation in many countries around the world.

CRISPR/Cas9 system

CRISPR/Cas9 is a powerful genome editing tool that has revolutionized the field of genetics and molecular biology (Doudna and Charpentier, 2014; Jabbar et al., 2021). It is a system derived from a natural bacterial immune system that allows for precise, targeted changes to the DNA of cells. The CRISPR/Cas9 system consists of two components: a guide RNA and a protein called Cas9. The guide RNA is designed to be complementary to a specific sequence of DNA, while the Cas9 protein acts like a pair of molecular scissors, cutting the DNA at the target site. The CRISPR/Cas9 system has many potential applications, including gene therapy for inherited genetic disorders, agriculture to develop crops with desirable traits, and research to study the function of genes and their role in disease. One of the main advantages of the CRISPR/Cas9 system is its efficiency and precision. It can be used to make very specific changes to the DNA of cells, and has been shown to be effective in a wide variety of cell types and organisms. Additionally, the CRISPR/Cas9 system is relatively easy to use and is much faster and more cost-effective than previous genome editing techniques.

Cas9 and Guide RNA

The CRISPR system is based on a naturally occurring immune defense mechanism that bacteria use to protect themselves from viruses. The CRISPR system consists of two main components: the Cas9 protein and a small RNA molecule called guide RNA (gRNA). The gRNA is designed to recognize and bind to a specific sequence of DNA in the genome, while the Cas9 protein (enzyme) acts as a pair of molecular scissors that cuts the DNA at the targeted location. The gRNA is typically engineered in the laboratory to target a specific gene or region of the genome. The schematics of CRISPR/Cas9 system is shown in ***Figure 1***.

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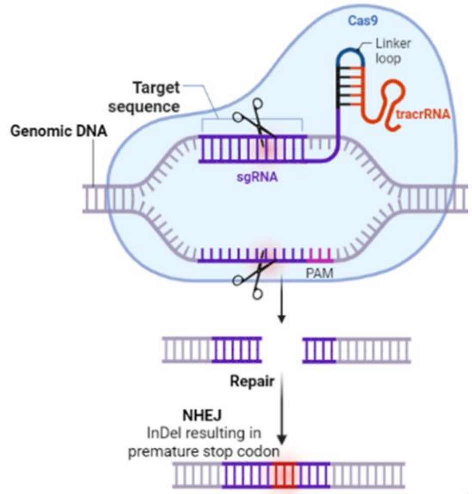

Figure 1: *Schematics of CRISPR/Cas9 Genetic Scissors*

Researchers often use software tools like CHOPCHOP (Montague *et al.*, 2014; Labun et al., 2021), which stands for "CRISPR/Cas9 Off-Target Prediction and Analysis Platform". CHOPCHOP is an online tool that enables users to design and analyze gRNAs for CRISPR-Cas9 gene editing experiments. The tool works by taking a user-defined DNA sequence and identifying potential gRNA target sites within that sequence. It then predicts the off-target effects of those gRNAs and provides information about potential mismatches and other factors that could affect the specificity of the gene editing experiment. Using CHOPCHOP, researchers can optimize the design of gRNAs to increase their efficiency and specificity, ultimately resulting in more accurate and effective gene editing experiments. The tool can also be used to analyze existing gRNAs to identify potential off-target effects and optimize their design.

When the gRNA is introduced into a cell along with the Cas9 protein, it binds to the DNA at the target site and forms a complex with Cas9. The Cas9 then cuts the DNA at that location, which triggers the cell's natural DNA repair machinery to either introduce a desired genetic change or disable the target gene altogether. The CRISPR/Cas9 system has revolutionized the field of gene editing and has numerous potential applications, including treating genetic disorders, developing new agricultural technologies, and engineering bacteria to produce biofuels.

CRISPR/Cas9 Gene Editing System in Livestock Animals

The CRISPR/Cas9 gene editing system has shown (***Figure 2***) promise as a tool for modifying the genomes of livestock animals, with potential applications in agriculture, biomedical research,

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and even disease prevention. By making targeted edits to an animal's genome, scientists can potentially enhance desirable traits such as disease resistance, growth rate, milk production, and meat quality, as well as reduce undesirable traits such as susceptibility to disease and susceptibility to environmental stress.

One potential application of the CRISPR/Cas9 system in livestock is to improve their health and welfare by introducing genetic modifications that make them more resistant to diseases or better suited to their environment. For example, researchers are exploring the use of CRISPR/Cas9 to develop pigs that are resistant to the Porcine Reproductive and Respiratory Syndrome virus, a major cause of disease in swine populations. Another application of CRISPR/Cas9 in livestock is in biomedical research, where genetically modified animals can be used as models for human diseases. For example, researchers have used CRISPR/Cas9 to create pigs that carry mutations associated with human cystic fibrosis, a genetic disorder that affects the lungs and digestive system. These pigs can be used to study the disease and test potential therapies. Additionally, researchers are exploring the use of CRISPR/Cas9 to create chickens that lay eggs with certain nutritional properties, such as eggs with lower cholesterol levels or higher levels of vitamins. There are also potential applications of CRISPR/Cas9 in aquaculture, where the technology could be used to increase the productivity and sustainability of fish and shellfish farming by introducing desirable traits such as disease resistance and improved growth rates. However, the use of CRISPR/Cas9 in livestock animals also raises

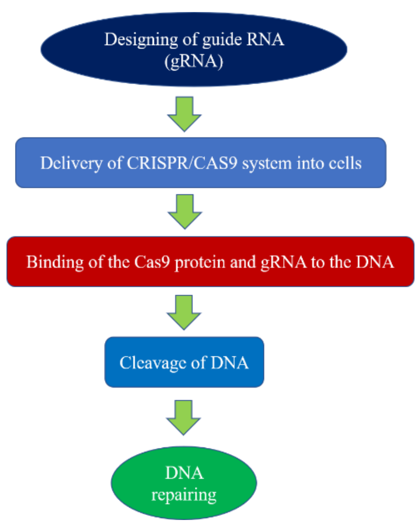

Figure 2: *Working of CRISPR/Cas9 gene editing system*

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ethical and safety concerns. There is a risk of unintended genetic changes and off-target effects, and there is a potential for animals to be harmed or for negative impacts on the environment. As a result, the use of CRISPR/Cas9 in animal agriculture is subject to regulations and oversight to ensure that it is safe, ethical, and environmentally responsible.

CRISPR/Cas9 and milk production

CRISPR/Cas9 may have the potential to improve milk production in livestock animals by introducing genetic modifications that increase milk yield and quality. One approach is to use CRISPR/Cas9 to introduce mutations into genes that affect milk production. For example, researchers are exploring the use of CRISPR/Cas9 to create cows that produce milk with higher levels of beta-casein, a protein that affects the quality of cheese and other dairy products. Other potential targets include genes that affect milk yield, milk fat content, and lactation efficiency. Another approach is to use CRISPR/Cas9 to modify the genetics of dairy cows in order to make them more resistant to diseases or better suited to their environment. This could include introducing genetic modifications that make cows more resistant to mastitis, a common bacterial infection in dairy cows that can reduce milk production and quality. CRISPR/Cas9 could also be used to introduce traits such as heat tolerance or feed efficiency, which could help cows adapt to changing environmental conditions.

CRISPR/Cas9 and meat production

CRISPR/Cas9 system can be used to introduce mutations into genes that affect meat production. For example, researchers are exploring the use of CRISPR/Cas9 to create pigs that produce leaner meat, with higher levels of unsaturated fatty acids, which are considered to be healthier for human consumption. Other potential targets include genes that affect meat yield, marbling, and tenderness. Another approach is to use CRISPR/Cas9 to modify the genetics of livestock animals in order to make them more resistant to diseases or better suited to their environment. This could include introducing genetic modifications that make animals more resistant to common diseases or environmental stressors, such as heat or cold temperatures. Myostatin is a protein that plays a critical role in regulating muscle growth and development in animals, including humans. In recent years, the CRISPR-Cas9 gene editing system has been used to explore the role of myostatin in animals and to develop strategies for enhancing muscle growth and performance. Several studies have shown that targeting the myostatin gene using CRISPR-Cas9 can result in significant increases in muscle growth and strength in a variety of animal species, including mice, pigs, and cattle. For example, in a study

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published in the journal Nature Communications in 2015, researchers used CRISPR-Cas9 to disrupt the myostatin gene in mice, resulting in a significant increase in muscle mass and strength compared to control mice. Similarly, in a study published in the journal Science in 2018, researchers used CRISPR-Cas9 to disrupt the myostatin gene in pigs, resulting in pigs with significantly increased muscle mass and strength compared to wild-type pigs. Overall, while the use of CRISPR-Cas9 to target the myostatin gene in animals is an active area of research, there are still many questions that need to be addressed before this technology can be used safely and responsibly in animal breeding or other applications.

CRISPR/Cas9 and animal reproduction

CRISPR/Cas9 has the potential to revolutionize reproductive biology by allowing scientists to modify the genes of embryos, gametes, and reproductive cells. One potential application of CRISPR/Cas9 in reproduction is in the treatment of genetic diseases. By editing the genome of an embryo, scientists could potentially correct disease-causing mutations and prevent the transmission of genetic diseases to future generations. This could be done through in vitro fertilization (IVF) and preimplantation genetic diagnosis (PGD), in which embryos are created in the laboratory and screened for genetic mutations before they are implanted into the mother's uterus. Another potential application of CRISPR/Cas9 in reproduction is in the development of new forms of contraception. By editing the genes that control fertility, scientists could potentially create new forms of birth control that are more effective and have fewer side effects than existing methods. Additionally, CRISPR/Cas9 could be used to study the genetics of reproduction and to better understand the mechanisms that control fertility and reproduction. This could lead to new treatments and interventions for infertility and other reproductive disorders. However, the use of CRISPR/Cas9 in reproduction raises significant ethical and safety concerns.

(1) CRISPR/Cas9 and estrus

Estrus is a reproductive cycle in female mammals that includes ovulation and the period of sexual receptivity. CRISPR/Cas9 has the potential to modify the genetics of animals and alter their reproductive cycles, including estrus. One potential application of CRISPR/Cas9 in estrus is to modify the genes that control the timing and duration of the reproductive cycle. For example, scientists could potentially use CRISPR/Cas9 to create cows that have shorter or more predictable estrus cycles, which would make it easier to manage breeding and improve the efficiency of dairy production. Another potential application of CRISPR/Cas9 in estrus is to create animals that are more resistant to diseases or stressors that can disrupt reproductive cycles. For example, scientists could use CRISPR/Cas9 to modify the genes that control immune function or stress responses, which could make animals more resistant to infections or environmental stressors that can affect reproductive health.

(2) CRISPR/Cas9 and pregnancy diagnosis

CRISPR/Cas9 has the potential to improve pregnancy diagnosis in livestock animals by allowing for more accurate and efficient methods of detecting pregnancy. One potential application of CRISPR/Cas9 in pregnancy diagnosis is through the modification of genes that are associated with pregnancy. For example, researchers have used CRISPR/Cas9 to create pigs that produce a fluorescent protein in their blood when they become pregnant, making it easier to detect pregnancy without the need for invasive procedures. Another potential application of CRISPR/Cas9 in pregnancy diagnosis is through the modification of genes that are associated with fetal development. For example, researchers have used CRISPR/Cas9 to create cows that produce a detectable number of pregnancy-associated glycoproteins (PAGs) in their blood, which are indicators of fetal development. CRISPR/Cas9 can also be used to modify genes that control the timing of pregnancy, such as those involved in the regulation of the estrous cycle. By modifying these genes, researchers could potentially create animals that are more efficient at conceiving and carrying pregnancies to term.

(3) CRISPR/Cas9 and semen sexing

CRISPR/Cas9 has the potential to improve semen sexing technology, which is used to produce offspring of a desired sex in livestock animals. One potential application of CRISPR/Cas9 in semen sexing is through the modification of genes that are associated with sex determination. For example, researchers have used CRISPR/Cas9 to modify the SRY gene (Kurtz et al., 2021), which is responsible for male sex determination, in mouse embryos. This could potentially be applied to livestock animals to create sperm cells that have a higher likelihood of producing offspring of a desired sex. Another potential application of CRISPR/Cas9 in semen sexing is through the modification of genes that are associated with fertility. By modifying these genes, researchers could potentially create sperm cells that are more efficient at fertilizing eggs, which could improve the success rate of semen sexing.

(4) CRISPR/Cas9 and embryo transfer technology

CRISPR/Cas9 and embryo transfer technology can be used together to create genetically

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modified organisms, including humans. This technology involves creating embryos through in vitro fertilization (IVF) and then using CRISPR/Cas9 to make precise edits to the DNA of those embryos. Embryo transfer technology allows scientists to transfer the edited embryos into a surrogate mother's womb, where they can grow and develop into a genetically modified offspring. This technology has the potential to treat and prevent genetic diseases, as well as to enhance desired traits in animals and crops. However, the use of CRISPR/Cas9 and embryo transfer technology in humans is controversial and raises ethical concerns. There are concerns about the potential for unintended consequences and the possibility of creating so-called "designer babies" with desired traits. Additionally, there are concerns about the long-term effects of editing the genome and the ethical implications of altering the genetic makeup of future generations. As a result, the use of CRISPR/Cas9 and embryo transfer technology in humans is heavily regulated and subject to strict ethical guidelines. Scientists and policymakers are continuing to debate the benefits and risks of these technologies and how they should be used in the future.

CRISPR/Cas9 and animal welfare

CRISPR/Cas9 has the potential to improve animal welfare in several ways, but there are also concerns that its use could have negative impacts on animal welfare (Singh and Ali, 2021). One potential application of CRISPR/Cas9 in animal welfare is to create animals that are more resistant to diseases, which could reduce the need for antibiotics and other treatments that can be stressful and harmful to animals. For example, researchers have used CRISPR/Cas9 to create pigs that are resistant to African swine fever, a highly contagious and deadly disease that can devastate pig populations. Another potential application of CRISPR/Cas9 in animal welfare is to create animals that are more efficient at converting feed into meat, which could reduce the environmental impact of meat production and improve the efficiency of animal agriculture. By reducing the amount of feed needed to produce meat, farmers could potentially reduce the amount of land needed for animal agriculture, which could reduce the destruction of natural habitats and wildlife. However, there are also concerns that the use of CRISPR/Cas9 in animal breeding and reproduction could have negative impacts on animal welfare. There is a risk of unintended genetic changes and off-target effects, which could lead to health problems and reduced quality of life for animals. In addition, there are ethical concerns related to the creation of animals that are genetically modified for human purposes. It is important to carefully evaluate the safety and efficacy of CRISPR/Cas9 interventions in animal breeding and

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reproduction, and to ensure that they are conducted in a responsible and ethical manner that considers the welfare of animals, as well as environmental and social impacts. Regulatory frameworks have been established in many countries to oversee and guide the use of CRISPR/Cas9 in animal research and agriculture.

CRISPR/Cas9 and animal ethics

The use of CRISPR/Cas9 in animal research raises a number of ethical considerations (Kang et al., 2017), particularly with regard to the welfare of the animals involved. One of the main concerns is that the genetic modifications made using CRISPR/Cas9 could cause unintended health effects or suffering in the animals. For example, the modifications could cause developmental abnormalities, disease susceptibility, or behavioral changes that could reduce the animal's quality of life. There is also concern about the potential for creating animals with traits that are not in their best interest, such as animals that are more aggressive or that grow too quickly, leading to health problems or suffering. Another ethical concern is the use of CRISPR/Cas9 to create animals with desirable traits, such as increased muscle mass or milk production. While such modifications could increase efficiency in animal agriculture, there is concern that they could contribute to the commodification of animals and reduce their value as living beings. In addition, there are concerns about the impact of CRISPR/Cas9-modified animals on the environment, particularly if they were to escape into the wild and potentially disrupt ecosystems. As a result of these ethical considerations, the use of CRISPR/Cas9 in animal research is subject to strict regulations and oversight to ensure that the welfare of the animals is protected and that the potential benefits of the research outweigh any potential harms. Researchers are also working to develop alternative methods that reduce the need for animal testing altogether, such as using in vitro methods or computer simulations.

CRISPR/Cas9 and evolution of next-generation theranostics

Theranostics is an emerging field of medicine that combines diagnostics and therapeutics into a single integrated approach. The goal of theranostics is to develop personalized treatments that are tailored to the individual patient, based on their unique genetic makeup and disease characteristics. CRISPR/Cas9 is already playing a significant role in the evolution of next-generation theranostics (Yadav et al., 2021). Its high precision and specificity make it an ideal tool for designing targeted therapies that can be customized for individual patients. One of the most promising applications of CRISPR/Cas9 in next-gen theranostics is the development of gene therapies that can treat genetic diseases by editing the patient's own DNA. By using CRISPR/Cas9 to target and correct

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genetic mutations that cause diseases like sickle cell anemia and cystic fibrosis, researchers are working to develop curative therapies that could transform the way these diseases are treated. In addition to gene therapy, CRISPR/Cas9 is also being used to develop new diagnostics that can rapidly and accurately detect diseases like cancer and infectious diseases. For example, researchers have developed CRISPR-based diagnostic tools like SHERLOCK that can detect specific sequences of DNA or RNA in samples like blood or saliva. These tools are highly sensitive and specific, and they could potentially be used to diagnose diseases in real-time, providing critical information for treatment decisions. Another promising application of CRISPR/Cas9 in next-gen theranostics is the development of precision medicines that are tailored to individual patients based on their genetic makeup. By using CRISPR/Cas9 to identify and target specific genetic mutations that drive disease, researchers are working to develop highly targeted therapies that can be customized for each patient, maximizing their effectiveness and minimizing side effects.

Overall, the evolution of next-gen theranostics is being driven by advances in technologies like CRISPR/Cas9 that enable precision medicine and personalized treatments. As these technologies continue to evolve and improve, we can expect to see a revolution in the way we diagnose and treat diseases.

CRISPR/Cas9 and loop mediated isothermal amplification

CRISPR/Cas9 and loop mediated isothermal amplification (LAMP) are two different technologies that are sometimes used together in diagnostic testing (Atçeken et al., 2022). LAMP is a molecular biology technique that allows the amplification of specific DNA or RNA sequences under isothermal conditions, meaning that the reaction can take place at a constant temperature. This is in contrast to traditional polymerase chain reaction (PCR) testing, which requires multiple temperature cycles. LAMP can be used to amplify DNA or RNA sequences from pathogens, such as viruses or bacteria, in a patient sample. This allows for rapid and sensitive detection of the pathogen, even at low levels. One way that LAMP is being used with CRISPR/Cas9 is to enhance the sensitivity and specificity of CRISPR-based diagnostic tests. LAMP can be used to amplify the target DNA sequence from a patient sample, and the amplified DNA can then be used as a substrate for the CRISPR/Cas9 system to detect and cleave the DNA at the target site. The CRISPR/Cas9 system is designed to only cleave the DNA if the target sequence is present, allowing for highly specific and sensitive detection of the pathogen. The combination of LAMP and CRISPR/Cas9 in diagnostic

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testing has the potential to be faster, more accurate, and more cost-effective than traditional diagnostic methods. However, as with all medical technologies, the use of LAMP and CRISPR/Cas9 in diagnostic testing is subject to regulatory oversight and clinical validation to ensure that it is safe, effective, and reliable.

The broader applications of the CRISPR/Cas9 system is shown in ***Figure 3***.

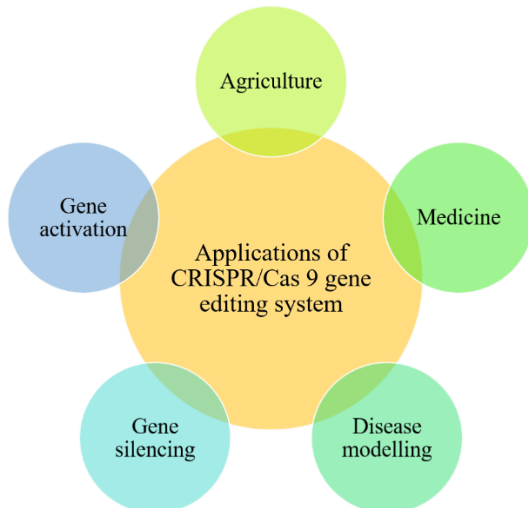

Figure 3: *Applications of CRISPR/Cas9 gene editing system*

Conclusion

In conclusion, the CRISPR-Cas9 gene editing system is a revolutionary tool that has transformed the field of genetics and has the potential to revolutionize medicine, agriculture, and many other fields. Overall, while CRISPR-Cas9 holds tremendous promise for advancing scientific and medical research, it is important that this technology is used responsibly and with careful consideration of the potential risks and benefits. Ongoing research and dialogue will be needed to ensure that this powerful tool is used safely and ethically, and that it benefits society as a whole.

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