

Review Article

An Overview of CRISPR-Cas9 Technology

Kaushik Bharati**How to cite this article:***Kaushik Bharati*. An Overview of CRISPR-Cas9 Technology. Ind J Genet Mol Res. 2025; 14(1): 00-00.**ABSTRACT**

CRISPR-Cas9 is a Nobel Prize winning groundbreaking technology that allows precise editing of an organism's genome, including that of humans. This technology can specifically modify target DNA sequences, thereby improving its action. The Cas9 enzyme and guide RNA are vital components of the CRISPR-Cas9 complex. The two main mechanisms responsible for its action are non-homologous end joining (NHEJ) and homology-directed recombination (HDR). There are several strategies that can be employed to deliver the CRISPR components into target cells. These include viral vectors, lipofection, electroporation, microinjection, and using nanoparticles. This robust technology has important applications in medicine and agriculture.

KEYWORDS

• CRISPR-Cas9, • Gene editing, • Genome, • Guide RNA, • Microinjection, • Viral vector, • Lipofection, • Nanoparticle, • Electroporation.

INTRODUCTION

Currently, there are numerous challenges in the areas of healthcare, agriculture, and environment, among others. These areas can benefit from the evolving CRISPR-Cas9 technology, which can be judiciously used to add, delete, or modify specific genetic sequences in an organism's genome by the process of gene editing.

What is CRISPR-Cas9?¹

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a powerful gene-editing tool that allows researchers to modify specific DNA sequences in an organism's genome, including that of humans. It has the potential to open-up new horizons in genetic research, biotechnology, and medicine.

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The Cas9 enzyme is at the core of CRISPR, which makes-up the CRISPR-Cas9 complex. It has its origins in the bacterial immune defence mechanisms against viruses. Besides the CRISPR-Cas9 technology, there are a couple of others, including CRISPR-Cas12a and CRISPR-Cas13 that have unique plus-points.

The CRISPR technology is not only a major milestone, but also a paradigm shift in medical research. CRISPR-Cas9 is a unique technique for making highly specific modifications to DNA, with razor-sharp accuracy. This technology is increasingly being used to treat genetic diseases, improve food crops, accelerate cutting-edge research, among other applications.

Mechanism of Action of CRISPR-Cas9²

The mechanism of action of CRISPR-Cas9 is briefly highlighted below:

CRISPR-Cas9 Targets Specific DNA Sequences

The essential component of CRISPR-Cas9 is the Cas9 enzyme and a guide RNA (gRNA). The gRNA's sequence exactly matches a specific DNA sequence in the target gene, thereby targeting the Cas9 enzyme to the particular location. After binding to the complementary DNA, the gRNA guides the Cas9 enzyme to

the specific position in the genome where the gene editing is to be done. This arises from the specific base-pairing between the gRNA and the target DNA sequence, allowing the targeting of virtually any gene with high accuracy.

The Cas9 Enzyme and gRNA Have Specific Functions

The Cas9 enzyme is activated by binding to the gRNA and then acts like a molecular scissor to cut the target DNA. The activated Cas9 enzyme then binds to the matching genomic sequence that immediately precedes 3-nucleotide protospacer adjacent motif (PAM) sequence. PAM is a short DNA sequence that is vital for cleaving the target DNA. Once the DNA is cleaved, the de novo DNA repair mechanisms are activated. There are two major DNA repair pathways, which include non-homologous end joining (NHEJ) and homology-directed recombination (HDR). NHEJ can result in insertions or deletions (indels) at the breakage site, which can hinder the function of the target gene, often resulting in its inactivation. Alternately, HDR is much more precise, as it uses a repair template to insert or replace DNA sequences (Fig. 1).

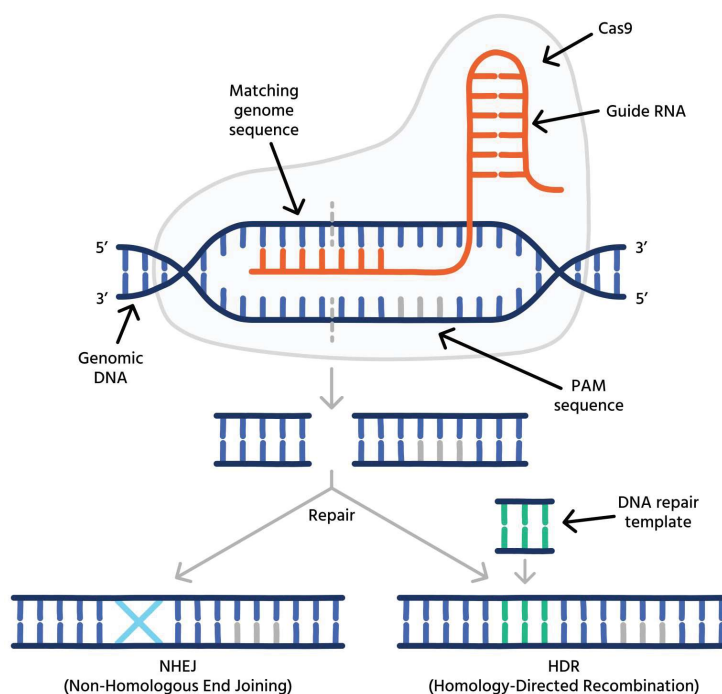


Fig. 1: CRISPR-Cas9 mechanism

History of Development of CRISPR-Cas9 Technology³

Initially, studying the bacterial defence mechanisms led to the discovery of the CRISPR-Cas9 technology, which was then slowly transformed into the revolutionary gene-editing tool, as we know it today. The path of discovery began in the late 1980s and progressed with the passage of time. This culminated in groundbreaking discoveries on CRISPR-Cas9 in the 2000s. Notably, CRISPR-Cas9's functionality was elucidated by Francisco Mojica and Ruud Jansen at this time. They found that CRISPR-Cas9 plays a critical

role in generating genetic memory of previous pathogenic infections.

Notably, during this period CRISPR-associated genes encoding Cas9 and similar proteins were discovered. This transformed CRISPR-Cas9 into a robust gene-editing tool, enabling researchers to harness the power of the inherent defence mechanism for specific DNA modification.

Major Milestones in CRISPR-Cas9 Research⁴

The major milestones during the discovery pathway of the CRISPR-Cas9 technology are briefly highlighted below:

Year	Milestone
2012	Jennifer Doudna and Emmanuelle Charpentier discovered the programmable nature of CRISPR-Cas9 for targeted DNA cleavage.
2013	Feng Zhang demonstrated CRISPR-Cas9's ability to edit mice and human genes.
2015	Demonstration of CRISPR-Cas9's ability to edit plant genes.
2017	Demonstration of CRISPR-Cas9's ability to correct genetic mutations in human embryos.
2020	Nobel Prize in Chemistry awarded to Jennifer Doudna and Emmanuelle Charpentier for their contributions to developing CRISPR-Cas9 technology.
2023	USFDA approved Casgevy, the first CRISPR-Cas9-based drug for treating sickle cell disease, manufactured by Vertex Pharmaceuticals and CRISPR Therapeutics

The Pros and Cons of CRISPR-Cas9 Technology⁵

Pros	Cons
High specificity	Potential for exhibiting off-target effects
Ease of designing	Difficulty in delivering CRISPR-Cas9 to certain cell types
Capability to edit multiple genes simultaneously	Ethical issues regarding genetic modifications or gene integration

CRISPR-Cas9 Delivery Strategies⁶

There are several strategies for delivering CRISPR components, which are briefly highlighted below:

- **Viral Vectors:** In this approach, a harmless virus carries the CRISPR components and delivers them into target cells. Some examples include lentiviruses and adenoviruses.
- **Nanoparticles:** These are nano-sized particles, having a diameter ranging between 1 and 100 nanometers. These can be constructed using various materials that have unique physical and chemical properties. For example, lipid nanoparticles encapsulating CRISPR components are excellent delivery vehicles. Other types may be non-lipid-based, one of the most popular being gold nanoparticles.

- **Lipofection:** In this approach, liposomes are used to deliver the CRISPR components. These encapsulate the CRISPR components and attach to the surface of cells and deliver the contents.
- **Micronjection:** In this technique, a microneedle is used to directly inject the CRISPR components into cells or embryos, making it a highly precise delivery mechanism.
- **Electroporation:** In this approach, an electrical current is used to create transient pores in the cell membrane, so that the CRISPR components can enter with ease.

Applications of CRISPR-Cas9 Technology⁷

The applications of CRISPR-Cas9 fall under two major categories, namely, medicine and agriculture, which are briefly highlighted below:

Medical Applications

- **Treatment of Genetic Disorders and Cancer:** CRISPR-Cas9 has important applications in the area of genetic disorders and cancer. Some of the genetic disorders in which the CRISPR-Cas9 technology has proved to be useful, include cystic fibrosis, muscular

dystrophy, and sickle cell anemia. This technology can now target and repair defective genes, thereby holding promise to cure previously incurable diseases.

CRISPR-Cas9 has also succeeded in deciphering the genetic basis of oncogenesis that could pave the way to eventually developing a cure for this dreaded disease. Notably, gene knockouts have been utilized for pinpointing critical genes responsible for cancer cell survival and their metastasis. This will help to design drugs for targeted therapies, minimizing side effects arising from off-target effects, thereby increasing efficacy.

- **HIV Therapy:** CRISPR holds promise for developing therapeutics against human immunodeficiency virus (HIV). The CRISPR-Cas9 technology has succeeded in eliminating HIV from infected human and mice cells, which is a major breakthrough in developing a potential cure.
- **Immunotherapy:** The power of the CRISPR-Cas9 technology can be harnessed for developing immunotherapies by engineering T-cells so that they can fight diseases more effectively. Studies have shown that this approach is feasible for treating various types of cancer.
- **Drug Development:** The CRISPR-Cas9 platform can be used for accelerating drug development. Novel drug candidates can be quickly identified and validated using this technology. Moreover, creation of genetic knockouts through gene-editing can provide insights into potential drug targets. The CRISPR-Cas9 technology can also be coupled to high-throughput screening (HTS) for identifying gene mutations and their effects on disease processes. This approach could expedite the development of cost-effective targeted therapies.

Agricultural Applications

The CRISPR-Cas9 technology is being utilized in the agricultural sector, which is briefly highlighted below:

- **Producing High-Quality Crops:** The CRISPR-Cas9 technology is capable of increasing the nutritional value and pest resistance of food crops. Gene

editing of particular traits makes the crops more resilient to climatic changes. This technology can also be used to fortify crops with essential vitamins and minerals to combat malnutrition in many low-income countries.

- **Reducing Carbon Footprint:** The CRISPR-Cas9 technology can help to engineer plants that neutralize greenhouse gas emissions. It can also increase carbon fixation and reduce carbon dioxide levels in the air.
- **Improving Plant Physiology:** CRISPR-Cas9 can edit genes of organelles, such as mitochondria and chloroplasts. For example, modification of chloroplasts through targeted gene editing can enhance photosynthesis. This has the potential to dramatically improve crop efficiency and productivity. Similarly, editing the mitochondrial genome can optimize energy utilization and stress resistance in crop plants.
- **Increasing Pest Resistance by Engineering Crops:** The traditional chemical pesticides have many limitations. CRISPR-Cas9 can help to engineer crops that makes them naturally resistant to pests. This technology has now made it possible to engineer wheat and tomato crops resistant to fungal infections.
- **Rearing Healthy Livestock:** CRISPR-Cas9 is also positively impacting livestock production. Targeted gene editing can enhance the production of healthier farm animals, including cattle, goats and sheep, which are used for meat and dairy products. Gene editing can also enhance their resistance against various diseases, thereby enhancing overall health and eliminating the need for antibiotic use. The power of CRISPR-Cas9 can also be utilized for improving milk production in domestic farm animals and increasing muscle mass in poultry to improve the yield and quality of the meat.

CONCLUSION

The CRISPR-Cas9 platform is a ground breaking genetic engineering tool that is revolutionizing medical research. It can edit genes with high precision and efficiency and has opened new avenues for researchers to

explore gene function, rectify genetic defects, and develop new therapies for genetic disorders. The technology holds tremendous potential for applications in agriculture, medicine, and biotechnology. However, it has major ethical concerns with regard to human germline editing that is likely to have long-term personal and societal consequences. Therefore using the technology judiciously and prudently will be instrumental while exploring its future potential.

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