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REVIEW

# Mechanism and Applications of CRISPR/Cas-9-Mediated Genome Editing



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**Abstract:** Clustered regularly interspaced short palindromic repeat (CRISPR) and their associated protein (Cas-9) is the most effective, efficient, and accurate method of genome editing tool in all living cells and utilized in many applied disciplines. Guide RNA (gRNA) and CRISPR-associated (Cas-9) proteins are the two essential components in CRISPR/Cas-9 system. The mechanism of CRISPR/Cas-9 genome editing contains three steps, recognition, cleavage, and repair. The designed sgRNA recognizes the target sequence in the gene of interest through a complementary base pair. While the Cas-9 nuclease makes double-stranded breaks at a site 3 base pair upstream to protospacer adjacent motif, then the double-stranded break is repaired by either non-homologous end joining or homology-directed repair cellular mechanisms. The CRISPR/Cas-9 genome-editing tool has a wide number of applications in many areas including medicine, agriculture, and biotechnology. In agriculture, it could help in the design of new grains to improve their nutritional value. In medicine, it is being investigated for cancers, HIV, and gene therapy such as sickle cell disease, cystic fibrosis, and Duchenne muscular dystrophy. The technology is also being utilized in the regulation of specific genes through the advanced modification of Cas-9 protein. However, immunogenicity, effective delivery systems, off-target effect, and ethical issues have been the major barriers to extend the technology in clinical applications. Although CRISPR/Cas-9 becomes a new era in molecular biology and has countless roles ranging from basic molecular researches to clinical applications, there are still challenges to rub in the practical applications and various improvements are needed to overcome obstacles.

**Keywords:** CRISPR, Cas-9, sgRNA, gene-editing, mechanism, applications

## Background

Genome editing is a type of genetic engineering in which DNA is deliberately inserted, removed, or modified in living cells.[1] The name CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) refers to the unique organization of short, partially repeated DNA sequences found in the genomes of prokaryotes. CRISPR and its associated protein (Cas-9) is a method of adaptive immunity in prokaryotes to defend themselves against viruses or bacteriophages.[2] Japanese scientist Ishino and his team accidentally found unusual repetitive palindromic DNA sequences interrupted by spacers in Escherichia coli while analyzing a gene for alkaline phosphatase first discovered CRISPR in 1987. However, they did not ascertain its biological function. In 1990, Francisco Mojica identifies similar sequences in other prokaryotes and he named CRISPR, yet the functions of these sequences were a mystery.[3] Later on in 2007, a CRISPR was experimentally conferred as a key element in the adaptive immune system of prokaryotes against

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viruses. During the adaptation process, bacterial cells become immunized by the insertion of short fragments of viral DNA (spacers) into a genomic region called the CRISPR array. Hence, spacers serve as a genetic memory of previous viral infections.[4] The CRISPR defense mechanism protects bacteria from repeated viral attacks via three basic stages: adaptation (spacer acquisition), crRNA synthesis (expression), and target interference. CRISPR loci are an array of short repeated sequences found in chromosomal or plasmid DNA of prokaryotes. Cas gene is usually found adjacent to CRISPR that codes for nuclease protein (Cas protein) responsible to destroy or cleave viral nucleic acid.[5]

Before the discovery of CRISPR/Cas-9, scientists were relied on two gene-editing techniques using restriction enzymes, zinc finger nucleases (ZFN) and Transcription activator-like effector nucleases (TALENs).[6] ZFN has a zinc finger DNA binding domain used to bind a specific target DNA sequence and a restriction endonuclease domain used to cleave the DNA at the target site. TALENs are also composed of DNA binding domain and restriction domain like ZFN but their DNA binding domain has more potential target sequence than the ZFN gene-editing tool. In both cases, the difficulty of protein engineering, being expensive, and time-consuming were the major challenges for researchers and manufacturers.[6,7] The development of a reliable and efficient method of a gene-editing tool in living cells has been a long-standing goal for biomedical researchers. After figuring out the CRISPR mechanism in prokaryotes, scientists understood that it could have beneficial use in humans, plants, and other microbes. It was in 2012 that Doudna, J, and Charpentier, E discovered CRISPR/Cas-9 could be used to edit any desired DNA by just providing the right template.[8] Since then, CRISPR/Cas-9 becomes the most effective, efficient, and accurate method of genome editing tool in all living cells and utilized in many applied disciplines.[9] Thus, this review aims to discuss the mechanisms of genome editing mediated by CRISPR/Cas-9 and to highlight its recent applications as one of the most important scientific discoveries of this century, as well as the current barriers to the transformation of this technology.

## Components of CRISPR/Cas-9

Based on the structure and functions of Cas-proteins, CRISPR/Cas system can be divided into Class I (type I, III, and IV) and Class II (type II, V, and VI). The class I systems consist of multi-subunit Cas-protein complexes, while the class II systems utilize a single Cas-protein. Since the structure of type II CRISPR/Cas-9 is relatively simple, it has been well studied and extensively used in genetic engineering.[10] Guide RNA (gRNA) and CRISPR-associated (Cas-9) proteins are the two essential components in CRISPR/Cas-9 system. The Cas-9 protein, the first Cas protein used in genome editing was extracted from *Streptococcus pyogenes* (SpCas-9). It is a large (1368 amino acids) multi-domain DNA endonuclease responsible for cleaving the target DNA to form a double-stranded break and is called a genetic scissor.[11] Cas-9 consists of two regions, called the recognition (REC) lobe and the nuclease (NUC) lobe. The REC lobe consists of REC1 and REC2 domains responsible for binding guide RNA, whereas the NUC lobe is composed of RuvC, HNH, and Protospacer Adjacent Motif (PAM) interacting domains. The RuvC and HNH domains are used to cut each single-stranded DNA, while PAM interacting domain confers PAM specificity and is responsible for initiating binding to target DNA.[12] Guide RNA is made up of two parts, CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA). The crRNA is an 18–20 base pair in length that specifies the target DNA by pairing with the target sequence, whereas tracrRNA is a long stretch of loops that serve as a binding scaffold for Cas-9 nuclease. In prokaryotes, the guide RNA is used to target viral DNA, but in the gene-editing tool, it can be synthetically designed by combining crRNA and tracrRNA to form a single guide RNA (sgRNA) in order to target almost any gene sequence supposed to be edited.[11]

## Mechanisms of CRISPR/CAS-9 Genome Editing

The mechanism of CRISPR/Cas-9 genome editing can be generally divided into three steps: recognition, cleavage, and repair.[13] The designed sgRNA directs Cas-9 and recognizes the target sequence in the gene of interest through its 5′crRNA complementary base pair component. The Cas-9 protein remains inactive in the absence of sgRNA. The Cas-9 nuclease makes double-stranded breaks (DSBs) at a site 3 base pair upstream to PAM.[14] PAM sequence is a short (2–5 base-pair length) conserved DNA sequence downstream to the cut site and its size varies depending on the bacterial species. The most commonly used nuclease in the genome-editing tool, Cas-9 protein recognizes the PAM sequence at 5′-NGG-3′ (N can be any nucleotide base). Once Cas-9 has found

a target site with the appropriate PAM, it triggers local DNA melting followed by the formation of RNA-DNA hybrid, but the mechanism of how Cas-9 enzyme melts target DNA sequence was not clearly understood yet. Then, the Cas-9 protein is activated for DNA cleavage. HNH domain cleaves the complementary strand, while the RuvC domain cleaves the non-complementary strand of target DNA to produce predominantly blunt-ended DSBs. Finally, the DSB is repaired by the host cellular machinery.[11,15]

### Double-Stranded Break Repair Mechanisms

Non-homologous end joining (NHEJ), and homology-directed repair (HDR) pathways are the two mechanisms to repair DSBs created by Cas-9 protein in CRISPR/Cas-9 mechanism.[16] NHEJ facilitates the repair of DSBs by joining DNA fragments through an enzymatic process in the absence of exogenous homologous DNA and is active in all phases of the cell cycle. It is the predominant and efficient cellular repair mechanism that is most active in the cells, but it is an error-prone mechanism that may result in small random insertion or deletion (indels) at the cleavage site leading to the generation of frameshift mutation or premature stop codon.[17] HDR is highly precise and requires the use of a homologous DNA template. It is most active in the late S and G2 phases of the cell cycle. In CRISPR-gene editing, HDR requires a large amount of donor (exogenous) DNA templates containing a sequence of interest. HDR executes the precise gene insertion or replacement by adding a donor DNA template with sequence homology at the predicted DSB site.[16,17]

## Applications of CRISPR/CAS-9

In just a few years of its discovery, the CRISPR/Cas-9 genome editing tool has already being explored for a wide number of applications and had a massive impact on the world in many areas including medicine, agriculture, and biotechnology. In the future, researchers hope that this technology will continue to advance for treating and curing diseases, develop more nutritious crops, and eradicating infectious diseases.[18] Highlights for some of the recent CRISPR/Cas-9 applications and clinical trials being investigated are discussed below.

### Role in Gene Therapy

More than 6000 genetic disorders have been known so far. But the majority of the diseases lack effective treatment strategies.[19] Gene therapy is the process of replacing the defective gene with exogenous DNA and editing the mutated gene at its native location. It is the latest development in the revolution of medical biotechnology. From 1998 to August 2019, 22 gene therapies including the novel CRISPR/Cas-9 have been approved for the treatment of human diseases.[20]

Since its discovery in 2012, CRISPR/Cas-9 gene editing has held the promise of curing most of the known genetic diseases such as sickle cell disease, β-thalassemia, cystic fibrosis, and muscular dystrophy.[21,22] CRISPR/Cas-9 for targeted sickle cell disease (SCD) therapy and β-thalassemia have been also applied in clinical trials.[23] SCD is an autosomal recessive genetic disease of red blood cells, which occurs due to point mutation in the β-globin chain of hemoglobin leading to sickle hemoglobin (HbS). During the deoxygenation process, HbS polymerization leads to severe clinical complications like hemolytic anemia.[24] Either direct repairing the gene of hemoglobin S or boosting fetal γ-globin are the two main approaches that CRISPR/Cas-9 is being used to treat SCD.[25] However, the most common method used in a clinical trial is based on the approach of boosting fetal hemoglobin. First bone marrow cells are removed from patients and the gene that turns off fetal hemoglobin production, called B-cell Lymphoma 11A (BCL11A) is disabled with CRISPR/Cas-9. Then, the gene-edited cells are infused back into the body.[26] BCL11A is a 200 base pair gene found on chromosome 2 and its product is responsible to switch γ-globin into the β-globin chain by repressing γ-globin gene expression.[27] Once this gene is disabled using CRISPR/Cas-9, the production of fetal hemoglobin containing γ-globin in the red blood cells will increase, thereby alleviating the severity and manifestations of SCD.[28]

Scientists have been also investigating CRISPR/Cas-9 for the treatment of cystic fibrosis. The genetic mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) gene decreases the structural stability and function of CFTR protein leading to cystic fibrosis.[29] CFTR protein is an anion channel protein regulated by protein kinase-A, located at the apical surface of epithelial cells of the lung, intestine, pancreas, and reproductive tract.[30] Although there is no cure for cystic fibrosis, symptom-based therapies (such as antibiotics, bronchodilators, and mucus thinning medications) and CFTR modulating drugs have become the first-line treatments to relieve symptoms and reduce the risk of complications.[31] Currently, gene manipulation technologies and molecular targets are also being explored. The use of CRISPR/Cas-9 technology for

genome editing has great potential, although it is in the early stages of development.[32] In 2013, researchers culture intestinal stem cells from two cystic fibrosis patients and corrected the mutation at the CFTR locus resulting in the expression of the correct gene and full function of the protein. Since then, the potential utility of the application of CRISPR/Cas-9 for cystic fibrosis was established.[33] Furthermore, Duchenne muscular dystrophy (DMD), which is caused by a mutation in the dystrophin gene and characterized by muscle weakness, has been successfully corrected by CRISPR/Cas-9 in patient-induced pluripotent stem cells.[34] Despite considerable efforts, the treatment available for DMD remains supportive rather than curative. Currently, several therapeutic approaches (gene therapy, cell therapy, and exon skipping) have been investigated to restore the expression of dystrophin in DMD muscles.[35,36] Deletion/excision of intragenic DNA and removing the duplicated exon by CRISPR/Cas-9 are the new and promising approaches in correcting the DMD gene, which restores the expression of dystrophin protein.[37]

Moreover, the latest researches show that the CRISPR/Cas-mediated single-base editing and prime editing systems can directly install mutations in cellular DNA without the need for a donor template. The CRISPR/Cas-base editor and prime editor system do not produce DSB, which reduces the possibility of indels that are different from conventional Cas-9.[38] So far, two types of base editors have been developed: cytosine base editor (CBE) and adenine base editor (ABE).[39] The CBE is a type of base editor composed of cytidine deaminase fused with catalytically deficient or dead Cas-9 (dCas-9). It is one of the novel gene therapy strategies that can produce precise base changes from cytidine (C) to thymidine (T).[40] However, the target range of the CBE base editor is still restricted by PAM sequences containing G, T, or A bases. Recently, a more advanced fidelity and efficiency base editor called nNme2-CBE (discovered from *Neisseria meningitides*) with expanded PAM compatibility for cytidine dinucleotide has been developed in both human cells and rabbits embryos.[41] The ABE uses adenosine deaminase fused to dCas-9 to correct the base-pair change from adenosine (A) to guanosine (G).[38] Overall, single-base editing through the fusion of dCas-9 to cytidine deaminase or adenosine deaminase is a safe and efficient method to edit point mutations. But both base editors can only fix four-transition mutations (purine to purine or pyrimidine to pyrimidine).[42] To overcome this shortcoming, the most recent member of the CRISPR genome editing toolkit called Prime Editor (PE) has been developed to extend the scope of DNA editing beyond the four types of transition mutations.[43] PE contains Cas-9 nickase fused with engineered reverse transcriptase and multifunctional primer editing guide RNA (pegRNA). The pegRNA recognizes the target nucleotide sequence; the Cas-9 nickase cuts the non-complementary strand of DNA three bases upstream from the PAM site, exposing a 3′-OH nick of genomic DNA. The reverse transcriptase then extends the 3′ nick by copying the edit sequence of pegRNA. Hence, PE not only corrects all 12 possible base-to-base transitions, and transversion mutations but also small insertion and deletion mutations in genetic disorders.[44]

## Therapeutic Role of CRISPR/Cas-9

The first CRISPR-based therapy in the human trial was conducted to treat patients with refractory lung cancer. Researchers first extract T-cells from three patient's blood and they engineered them in the lab through CRISPR/Cas-9 to delete genes (*TRAC*, *TRBC*, and *PD-1*) that would interfere to fight cancer cells. Then, they infused the modified T-cells back into the patients. The modified T-cells can target specific antigens and kill cancer cells. Finally, no side effects were observed and engineered T-cells can be detected up to 9 months of post-infusion.[45] CRISPR/Cas-9 gene-editing technology could also be used to treat infectious diseases caused by microorganisms.[46] One focus area for the researchers is treating HIV, the virus that leads to AIDS. In May 2017, a team of researchers from Temple University demonstrated that HIV-1 replication can be completely shut down and the virus eliminated from infected cells through excision of HIV-1 genome using CRISPR/Cas-9 in animal models.[47] In addition to the approach of targeting the HIV-genome, CRISPR/Cas-9 technology can also be used to block HIV entry into host cells by editing chemokine co-receptor type-5 (*CCR5*) genes in the host cells. For instance, an in vitro trial conducted in China reported that genome editing of CCR5 by CRISPR/Cas-9 showed no evidence of toxicity (infection) on cells and they concluded that edited cells could effectively be protected from HIV infection than unmodified cells.[48]

## Role in Agriculture

As the world population continues to grow, the risk of shortage in agricultural resources is real. Hence, there is a need for new technologies for increasing and improving

natural food production. CRISPR/Cas-9 is an existing addition to the field since it has been used to genetically modify foods to improve their nutritional value, increase their shelf life, make them drought-tolerant, and enhance disease resistance.[18] There are generally three ways that CRISPR is solving the world's food crisis. It can restore food supplies, help plants to survive in hostile conditions, and could improve the overall health of the plants.[49]

### Role in Gene Activation and Silencing

Beyond genome editing activity, CRISPR/Cas-9 can be used to artificially regulate (activate or repress) a certain target of a gene through advanced modification of Cas-9 protein.[15] Researchers had performed an advanced modified Cas-9 endonuclease called dCas-9 nuclease by inactivating its HNH and RuvC domains. The dCas-9 nuclease lacks DNA cleavage activity, but its DNA binding activity is not affected. Then, transcriptional activators or inhibitors can be fused with dCas-9 to form the CRISPR/dCas-9 complex. Therefore, catalytically inactive dCas-9 can be used to activate (CRISPRa) or silence (CRISPRi) the expression of a specific gene of interest.[50] Moreover, the CRISPR/dCas-9 can be also used to visualize and pinpoint where specifically the gene of interest is located inside the cell (subcellular localization) by fusing a marker such as Green Fluorescent Proteins (GFP) with dCas-9 enzyme. This enables site-specific labeling and imaging of endogenous loci in living cells for further utilization.[51]

## Challenges for CRISPR/Cas-9 Application

Despite its great promise as a genome-editing system CRISPR/Cas-9 technology had hampered by several challenges that should be addressed during the process of application. Immunogenicity, lack of a safe and efficient delivery system to the target, off-target effect, and ethical issues have been the major barriers to extend the technology in clinical applications.[52] Since the components of the CRISPR/Cas-9 system are derived from bacteria, host immunity can elicit an immune response against these components. Researchers also found that there were both pre-existing humoral (anti-Cas-9 antibody) and cellular (anti-Cas-9 T cells) immune responses to Cas-9 protein in healthy humans. Therefore, how to detect and reduce the immunogenicity of Cas-9 protein is still one of the most important challenges in the clinical trial of the system.[53]

Safe and effective delivery of the components into the cell is essential in CRISPR/Cas-9 gene editing. Currently, there are three methods of delivering the CRISPR/Cas-9 complex into cells, physical, chemical, and viral vectors. Non-viral (physical and chemical) methods are more suitable for ex vivo CRISPR/Cas-9-based gene editing therapy.[54] The physical methods of delivering CRISPR/Cas-9 can include electroporation, microinjection, hydrodynamic injection, and so on. Electroporation applies a strong electric field to the cell membrane to temporarily increase the permeability of the membrane, allowing the CRISPR/Cas-9 complex to enter the cytoplasm of the target cell. However, the main limitation of this method is that it causes significant cell death.[55] Microinjection involves injecting the CRISPR/Cas-9 complex directly into cells at the microscopic level for rapid gene editing of a single cell. Nevertheless, this method also has several disadvantages such as cell damage, which is technically challenging and is only suitable for a limited number of cells.[56] The hydrodynamic injection is the rapid injection of a large amount of high-pressure liquid into the bloodstream of animals, usually using the tail vein of mice. Although this method is simple, fast, efficient, and versatile, it has not yet been used in clinical applications due to possible complications.[57] The chemical methods of CRISPR/Cas-9 delivery involves lipid and polymer-based nanoparticles.[58] Lipid nanoparticles/liposomes are spherical structures composed of lipid bilayers membrane and are synthesized in aqueous solutions using Lipofectamine-based reagents. The positively charged liposomes encapsulated with negatively charged nucleic acids thereby facilitate the fusion of the complex across the cell membrane into cells.[59] Polymeric nanoparticles, such as polyethyleneimine and poly-L-lysine, are the most widely used carriers of CRISPR/Cas-9 components. Like lipid nanoparticles, polymer-based nanoparticles can also transverse the complex in the membrane through endocytosis.[60]

Viral vectors are the natural experts for in vivo CRISPR/Cas-9 delivery.[61] Vectors, such as adenoviral vectors (AVs), adeno-associated viruses (AAVs), and lentivirus vectors (LVs) are currently being widely used as delivery methods due to their higher delivery efficiency relative to physical and chemical methods. Among them, AAVs are the most commonly used vectors due to their low immunogenicity and non-integration into the host cell genome compared to other viral vectors.[62] However, the limited virus cloning capacity and the large size of the Cas-9 protein remain a major problem. One strategy to tackle this hurdle is to package sgRNA and Cas-9 into separate AAVs and then co-transfect them into cells.

Recent methods employ a smaller strain of Cas-9 from *Staphylococcus aureus* (SaCas-9) instead of the more commonly used SpCas-9 to allow packaging of sgRNA and Cas-9 in the same AAVs.[54,61] Lately, the development of extracellular vesicles (EVs), for the in vivo delivery of CRISPR/Cas-9 to avoid some of the limitations of viral and non-viral methods has shown a great potential.[63]

The designed sgRNA will mismatch to the non-target DNA and can result in nonspecific, unexpected genetic modification, which is called the off-target effect.[57] The CRISPR/Cas-9 target efficiency is determined by the 20-nucleotide sequences of sgRNA and the PAM sequences adjacent to the target genome. It has been shown that more than three mismatches between the target sequence and the 20-nucleotide sgRNA can result in off-target effects.[64] The off-target effect can possibly cause harmful events such as sequence mutation, deletion, rearrangement, immune response, and oncogene activation, which limits the application of the CRISPR/Cas-9 editing system for therapeutic purposes.[65] To mitigate the possibility of CRISPR/Cas-9 off-target effect, several strategies have been developed, such as optimization of sgRNA, modification of Cas-9 nuclease, utilization of other Cas-variants, and the use of anti-CRISPR proteins.[66] Selecting and designing an appropriate sgRNA for the targeted DNA sequence is an important first step to reduce the off-target effect.[67] When designing sgRNA, strategies such as GC content, sgRNA length, and chemical modifications of sgRNA must be considered. Generally speaking, studies revealed that GC content of between 40% and 60%, truncated (short length of sgRNA), and incorporation of 2′-O-methyl-3′-phosphonoacetate in the sgRNA ribose-phosphate backbone are the preferred methods to increase genome editing efficiency of CRISPR/Cas-9.[67,68] Modifying the Cas-9 protein to optimize its nuclease specificity is another way to reduce off-target effects. For instance, mutating either one of the catalytic residues of Cas-9 nuclease (HNH and RuvC) will convert the Cas-9 into nickase that could only generate a single-stranded break instead of a blunt cleavage.[69] It has been reported that the use of the inactivated RuvC domain of Cas-9 with sgRNA can reduce the off-target effect by 100 to 1500 times.[70] Moreover, the nuclease Cas-12a (previously known as Cpf1) is a type V CRISPR/Cas system that provides high genome editing efficiency.[71] Unlike the CRISPR/Cas-9 system, CRISPR/Cas-12a can process pre-crRNA into mature crRNA without tracrRNA, thereby reducing the size of plasmid constructs. The Cas-12a protein recognizes a T-rich (5′-TTTN) PAM sequence instead of 5′-NGG and provides high accuracy at the target gene loci than Cas-9.[69] Recently, the use of multi-component Class I CRISPR proteins, such as CRISPR/Cas-3 and CRISPR/Cas-10 provides better genome editing efficiency than Cas-9.[72] The Cas-3 is an ATP-dependent nuclease/helicase that can delete a large part of DNA from the target site without prominent off-target effect. For instance, the DMD gene were repaired by Cas-3-mediated system in induced pluripotent stem cell.[73] The Cas-10 protein does not require the PAM sequence and can identify sequences even in the presence of point mutation.[72] Anti-CRISPR (Acr) proteins are phage derived small proteins that inhibit the activity of CRISPR/Cas system. They are a recently discovered method to reduce off-target effects of CRISPR/Cas-9.[74] From Acr proteins, AcrIIA4 specifically targets Cas-9 nuclease. AcrIIA4 mimics DNA and binds to the Cas-9 site, making impossible to perform further cleavage in area outside the target region.[75] Furthermore, CRISPR/Cas-9 gene editing has been challenged by ethics and safety all over the world. Since the technology is still in its infancy and knowledge about the genome is limited, many scientists restrain that it still needs a lot of work to increase its accuracy and make sure that changes made in one part of the genome do not have unforeseen consequences, especially in the application towards human trials.[52]

## Conclusions

CRISPR/Cas-9 system in nature is used to protect prokaryotes from invading viruses by recognizing and degrading exogenous genetic elements. CRISPR/Cas-9 gene editing is adopted from acquired immunity in prokaryotes and consists of two elements: guide RNA used to locate (bind) the target DNA to be edited and Cas-9, a protein that essentially cuts the DNA at the location identified by guide RNA. The fundamental part of the CRISPR/Cas-9 gene-editing process is the identification of the target gene that determines the phenotype of interest and designing the guide RNA. Now it becomes a new era in molecular biology and has countless roles ranging from basic molecular researches to clinical applications. Although tremendous efforts have been made, there are still some challenges to rub in the practical applications and various improvements are needed to overcome obstacles in order to assure its maximum benefit while minimizing the risk.

## Abbreviations

AAVs, adeno-associated viral vectors; ABE, adenine base editor; Acr, anti-CRSPR; AVs, adeno-viral vectors; ATP, adenosine tri-phosphate; BCL11A, B-cell lymphoma 11 A; CAS-9, CRISPR-associated protein-9; CBE, cytidine base editor; CCR5, chemokine receptor type 5; CFTR, cystic fibrosis conductance transmembrane receptor; CRISPR, clustered regularly interspaced short palindromic repeat; CrRNA, CRISPR ribonucleic acid; DMD, Duchenne muscular dystrophy; DNA, deoxyribonucleic acid; DSBs, double-stranded breaks; HDR, homology-directed repair; LVs, lentivirus vectors; NHEJ, non-homologous end Jjining; PAM, protospacer adjacent motif; PD-1, programmed cell death-1; RNA, ribonucleic acid; TALENs, transcriptionactivator like effector nucleases; TRAC, T-cell receptor alpha; TRBC, T-cell receptor beta; TracrRNA, trans-activating CRISPR ribonucleic acid; ZFNs, zinc finger nucleases.

## Ethics Approval and Consent to Participate

Not applicable.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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