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REVIEW ARTICLE

CRISPR-Cas9 system: A genome editing tool

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Abstract

The CRISPR-Cas9 system has become as a groundbreaking tool for modifying genomes, completely changing the field of genetic research and biotechnology. This review article outlines the historical background, constituents and operation of the CRISPR-Cas9 system, emphasizing its exceptional accuracy and adaptability in selectively modifying DNA sequences. Initially discovered as an adaptive immune system in prokaryotes, CRISPR-Cas9 has since been adapted for use in various species, enabling targeted gene editing through gene disruption, insertion and correction. The review examines the technical components of the CRISPR-Cas9 system, including the design of guide RNA, delivery methods and the potential for off-target effects. It also explores recent advancements aimed at enhancing the accuracy and efficiency of this technology. Furthermore, the article discusses the broad applications of CRISPR-Cas9 in fields such as cancer research, gene therapy and agricultural biotechnology, underscoring its potential to provide innovative solutions for genetic disorders and to improve crop resilience. In addition, the review discusses the ethical and regulatory considerations associated with genome editing, emphasizing the significance of responsible and judicious use of this powerful technology. By analysing current research and exploring future directions, this study aims to provide a comprehensive overview of the CRISPR-Cas9 system and its profound impact on science and medicine.

Keywords: biotechnology; CRISPR-Cas9; DNA sequences; gene therapy; genome editing

Introduction

CRISPR-Cas9 is an incredibly powerful tool for editing the genetic code of different organisms. Initially identified in microorganisms such as bacteria as a constituent of a highly adaptable immune system CRISPR-Cas9 and its modified versions have since been widely used to manipulate genomes and regulate gene expression (1). CRISPR-Cas9 has significantly improved the accuracy and effectiveness of illness detection, particularly in cancer diagnostics (2). Consequently, this approach enables us to identify the precise process of carcinogenesis, allowing us to quickly identify targets for drug development and perhaps build cell -based therapies. The subject of genome editing has rapidly advanced, resulting in development of accessible, versatile and cost-effective methodologies for scientific research. The CRISPR framework has become a widely used technology for genetic manipulation both in laboratory settings (*in vitro*) and in living organisms (*in vivo*) (3-7).

The Cas9 protein, derived from *Streptococcus pyogenes* (Sp. Cas9), is a central component of the CRISPR genome editing tool, sometimes referred to as CRISPR-Cas9. Cas9 is an RNA-guided nuclease that uses a single guide RNA (sgRNA) to direct complex to a particular region of the genome. Previous studies have significantly advanced our understanding of of the CRISPR-Cas9 system (8-10). The PAM-interacting domain of the Cas9 protein recognizes a

protospacer that is present close to PAM sequences, which is essential for the functionality of the Cas-sgRNA complex (11, 12). When Cas9 persuades successfully, it induces a double-strand break (DSB) by cleaving the non-complementary DNA strand. Contrary to the earlier belief that Cas9 creates a blunt-end cut, recent studies have demonstrated that it preferentially leaves a single-nucleotide overhang, four nucleotides upstream of the PAM sequence (13, 14). The DSB introduced by Cas9 is repaired by endogenous cellular mechanisms, including homology-directed repair (HDR) and non-homologous end joining (NHEJ) (Fig. 1). Through HDR, researchers can insert any desired sequence into the host genome by introducing an exogenous DNA template flanked by homologous arms (15-17).

The challenge of engineering custom DNA-binding proteins has been significantly overcome over the past decade, largely due to the development of CRISPR-Cas9 technology. Unlike traditional methods that rely on protein-DNA interactions, CRISPR-Cas9 achieves target specificity through nucleic acid base pairing. A study describes the CRISPR-Cas tool as a prokaryotic adaptive immunity approach that breaks down invasive nucleic acids (18). Microorganisms such as bacteria and archaea exhibit a diverse array of CRISPR-Cas systems, each with distinct components and mechanisms of action. Class 2 CRISPR-Cas systems employ single-effector proteins, whereas Class 1 systems consist of multiprotein effector complexes. To date, at least six types and 29 subtypes of CRISPR-Cas systems have been identified and this number continues to grow rapidly (19-21).

Targeting specificity in CRISPR-Cas systems is conferred by CRISPR RNA (crRNA) or, in experimental CRISPR-Cas9 systems, by guide RNA (gRNA) (Fig. 1). The Cas enzyme cleaves the target nucleic acid when the spacer region of the crRNA hybridizes with a complementary target sequence located adjacent to a protospacer adjacent motif (PAM), or a protospacer flanking sequence (PFS) in type VI systems. This enables site-specific cleavage by designing crRNAs with appropriate spacer sequences that align with target loci containing a PAM or a PFS. The identification and development of type II CRISPR-Cas9 structures, together with their advantageous utilization, have facilitated rapid acceptance and progress in a wide variety of applications, spanning from basic technical knowledge to translational research and medicine (22-24). These early successes have fueled ongoing efforts to discover novel CRISPR-Cas systems and to expand the scope of genome engineering.

This article highlights recent advancements in CRISPR -Cas tools for gene editing and epigenetic modification. It discusses a broad range of emerging features within the CRISPR-Cas platform and explores its therapeutic potential, which in currently under investigation in various scientific studies. Furthermore, it provides an in-depth analysis of studies that have used the CRISPR tool for genome editing in order to get a better understanding of its mechanism in both *in vitro* and *in vivo* systems. Additionally, the article examines innovations aimed at optimizing the CRISPR system for use as a cutting- edge therapeutic approach.

History and Discovery of CRISPR-Cas9

The DNA sequences of *Escherichia coli* bacteria were the first to be found containing clustered regularly interspaced short palindromic repeats, or CRISPR. It was first described by Ishino and associates from Osaka University in Japan in 1987 (25). During that period, the process of sequencing these DNA fragments was laborious and time-consuming, taking several months. The researchers who identified these sequences had limited knowledge about their origin or functional significance within the bacterial cell. During the early stages of CRISPR research, the biological function of the system remained unclear. Nonetheless, scientists had already begun to propose potential applications of CRISPR loci in medical research, particularly in bacterial genotyping. This approach was initially applied to *Mycobacterium tuberculosis* in 1993 and later to *Streptococcus pyogenes* in 1999 (26). The high variability of CRISPR loci among strains of the same harmful bacterial species, facilitating the discernment of bacterial strains in clinical settings (27).

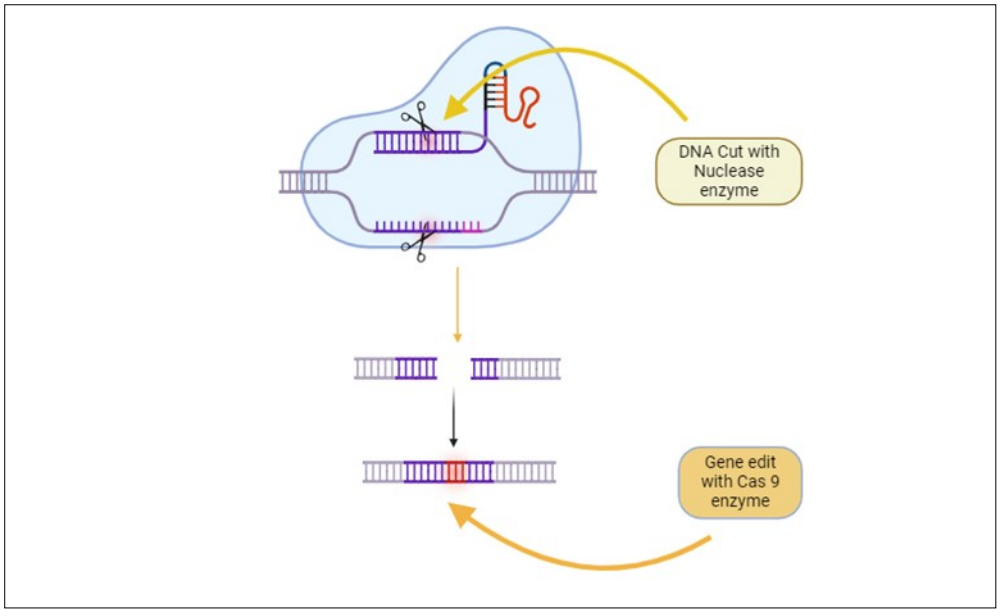

Fig. 1. Synthesis of toxic bioactive compounds by plants to mitigate environmental stresses.

A major breakthrough in understanding the biological function of CRISPR loci came in 1995, when Francisco Mojica from the University of Alicante (Spain) discovered similar structures in the archaeal genome of *Haloferax mediterranei* (28). The presence of such elements in two evolutionarily distant domains-bacteria and archaea-highlighted their likely functional importance and prompted further investigation. A study observed that the DNA repeats found in archaeal genomes resembled those previously identified in bacteria. In a pivotal 2005 publication proposed that these distinctive loci form part of a prokaryotic immune system and contain fragments of foreign DNA (29). That same year, two other independent research groups arrived at similar conclusions, marking the beginning of a vigorous phase of exploration into these extraordinary natural systems (30,31).

Consistent with the prokaryotic immune system hypothesis, viral DNA segments, known as "spacers," typically range from 17 to 84 bases in length. These spacers are separated by short palindromic repeats, which are 23 to 50 bases long (32). Furthermore, these spacers are organized into clusters located in intergenic regions of the genome (33). At first, it was hypothesized that this system would function via the process of RNA interference. However, several publications provided experimental evidence that the immune system of prokaryotes specifically targets foreign DNA, rather than mRNA (34). This discovery suggests that this system could be a valuable tool for genomic editing in laboratory settings. Subsequent research has shown that certain CRISPR systems are capable of functioning directly with RNA molecules (35, 36). Consequently, these systems can be employed to selectively disable specific transcripts within cells (37, 38).

Components of CRISPR/Cas9

Based on how Cas-proteins are arranged and function, the CRISPR/Cas system is categorized. Two primary classes may be used to classify it: Class I, which includes types I, III and IV and Class II, which includes types II, V and VI. In class II systems, a single Cas-protein is used, but in class I systems, Cas-protein complexes with several subunits occur. Among these, the type II CRISPR/Cas-9 has been extensively studied and used in genetic engineering because of its relatively simple structure (39).

The CRISPR/Cas9 system consists of two essential components: the Cas-9 protein and guide RNA (gRNA). Sourced from *Streptococcus pyogenes* (SpCas-9), the Cas-9 protein was the first Cas protein utilized in genome editing and is often referred to as a multi-domain DNA endonuclease, or “genetic scissors”. Cas9 introduces double-stranded break at the target DNA site, which consists of 1368 amino acids in total.

Structurally, the Cas9 protein comprises two major lobes: the recognition (REC) lobe and the nuclease (NUC) lobe. The REC lobe contains the REC1 and REC2 domains responsible for binding to the guide RNA. The NUC lobe includes the HNH, RuvC and PAM-interacting domains. The HNH and RuvC domains cleave the complementary and non -complementary strands of the DNA, respectively, while the PAM-interacting domain provides PAM sequence specificity and initiates the binding process with the target DNA (40).

Guide RNA is composed of two molecules: CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA). The crRNA is a short nucleic acid molecule, typically 18-20 base pairs in length and identifies the target DNA through complementary base pairing. In contrast, the tracrRNA is a longer molecule with several loops, serving as a structural scaffold required to activate the Cas9 nuclease. In prokaryotes, this guide RNA system enables precise targeting of viral DNA. For genome editing purposes, the crRNA and tracrRNA can be fused into a single guide RNA (sgRNA), a synthetic RNA molecule capable of targeting virtually any desired gene sequence with high specificity.

Proteins involved in CRISPR-Cas systems

It is sufficient to include spacers into the CRISPR cassettes using the proteins Cas1 and Cas2, which are found in most known CRISPR-Cas systems. These two proteins form a complex that is required for this modification process; Cas1's endonuclease activity is required for spacer integration, whereas Cas2 seems to have a non-enzymatic function (41). Encoding the highly conserved "data processing" module of CRISPR-Cas, the Cas1-Cas2 complex seems to operate somewhat independently of the other elements.

Subsequently, pre-crRNA is processed into guide crRNAs either by a specific RNA endonuclease complex or through an alternative mechanism involving bacterial RNase III in conjunction with an accessory RNA molecule (42). The resulting crRNA is then associated with one or more Cas proteins, depending on the system type-type II for the type II system and types I and III for their respective systems. These Cas proteins form an effector complex that accurately targets the corresponding DNA or RNA. This mechanism has been supported by the previous findings (43, 44). The effector complex in type I systems is known as Cascade (CRISPR-associated complex for antiviral defense) (45).

Genome editing

Specific DNA or RNA sequences may be attached to by proteins called CRISPR-Cas complexes, which are programmable. CreRNAs or gRNAs with spacers that match the target sequence are created in order to accomplish this binding. These proteins are appropriate for directed genome editing because they work as nucleases in addition to binding certain nucleic acid sequences.

Several CRISPR-Cas systems with unique characteristics such as PAM specificity, protein length and nuclease activity have been created for the purpose of genome editing. Furthermore, there has been significant progress in the formulation of techniques to identify both desired and undesired interactions, with a particular focus on enhancing the precision of the CRISPR-Cas based technologies.

CRISPR-Cas genes contribute for genome editing

The three CRISPR-Cas kinds can be clearly identified by the presence of three unique signature genes: Cas3 in type I systems, Cas9 in type II and Cas10 in type III (19). All three CRISPR-Cas types-with a few notable exceptions-have full sets of the parts required for the defence mechanism's key

phases. Fundamental concepts of the complexes' structure and operation have recently been found by in-depth investigation and study of the effector structures in several CRISPR-Cas system iterations. For type I and type III systems have contributed to a more comprehensive understanding of CRISPR-Cas system classification.

Cas3

Cas3 is a well-known type I system of the class 1 categorization in CRISPR-Cas frameworks. It consists of a complex called 'Course' and the endonuclease Cas3, which targets DNA (Fig. 2). Prior to recruiting Cas3 for a specific DNA grouping, the Cascade complex must first attach to DNA by recognizing PAM and spacer sequences (46-50). The course provides more website flexibility due to its unrestricted acceptance of PAM sequences (43). The presence of Cas3 leads to the creation of a single stranded break, followed by degradation of the target DNA via 3' to 5' exonuclease activity (8, 51, 52). Both the nickase activity and the helicase function of Cas3 are essential for the degradation of distant DNA in prokaryotes (49).

The Cas3 enzyme, known for its extraordinary cutting ability, is being used as an antibacterial tool. It does this by integrating with either local or foreign type I systems within bacterial genomes, leading to the destruction of the genomes and subsequent death of the cells (53). An inquiry into the reutilization of the nickase, helicase and exonuclease functions of Cas3 has the potential to stimulate novel uses in mammalian cells.

Cas9

Cas9 belongs to the class 2 type II CRISPR systems and is the most often used enzyme for genome editing. Specifically, *Streptococcus pyogenes* Cas9 (SpCas9) was the first to be extracted from prokaryotic cells and then modified for manipulating the genome in mammalian cells (54-56). It continues to be the most often used Cas9 enzyme. Upon recognition of the DNA target, SpCas9 often generates a blunt double-strand break (DSB)13 (Fig. 3). The process of DNA targeting by SpCas9 relies on a spacer that is 20 nucleotides long and a PAM sequence that is 5′-NGG (where N may represent any nucleotide) supported by studies (54, 57). Cas9 enzymes are directed by two RNA molecules: the CRISPR RNA (crRNA), which directs DNA targeting and the trans-activating crRNA (tracrRNA), which forms a complex with the crRNA and Cas9 to facilitate cleavage (41, 57).

The requirement for the 5′-NGG PAM limits SpCas9's targetable sites in the human genome to approximately one in every eight base pairs (24). To broaden the range of accessible target sites, rational engineering approaches have been employed to modify PAM recognition specificity (59, 60) (Table 1). One such engineered variant, xCas9, can recognize expanded PAM sequences, including 5′-NG, 5′-GAA and 5′-GAT (58).

Another important objective in developing Cas9 variants is to enhance target specificity. Multiple studies have demonstrated that engineered Cas9 proteins exhibit reduced off-target cleavage when delivered either as plasmid DNA or as ribonucleoprotein complexes (61-65). Recent advancements have further improved the precision of CRISPR-Cas systems by incorporating RNA clamps within the spacer region of guide RNAs. This modification maintains robust on-target activity while minimizing off-target interactions between crRNA or gRNA strands and non-target DNA (65, 66).

The discovery of Cas9 orthologues that recognize different PAM sequences has expanded the range of targetable genomic sites. For instance, *Streptococcus thermophilus* Cas9 recognizes the PAM 5′-NNAGAAW, where W denotes either adenine or thymine (8, 67), while *Neisseria meningitidis* Cas9 recognizes 5′-NNNNGATT (67). These orthologues have been employed for DNA targeting in both microbial and human cell systems. Additionally, the Cas9 enzyme from *Staphylococcus aureus* (SaCas9) recognizes the PAM 5′-NNGRRT (where R represents adenine or guanine) (68). Despite its smaller size-comprising 1,053 amino acids compared to SpCas9's 1,368-SaCas9 exhibits comparable genome editing efficiency. Its compact size makes it particularly suitable for delivery via size-constrained vectors such as adeno-associated viruses (AAV) (69).

A more compact Cas9 orthologue was recently

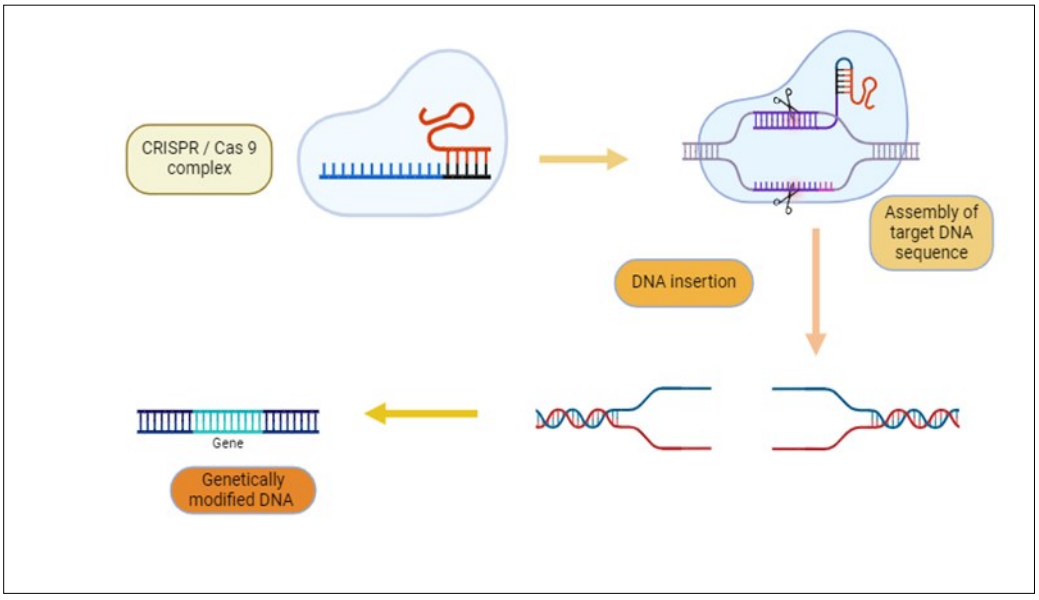

Fig. 2. Mechanism of CRISPR /Cas 9 system of DNA molecule.

identified in *Campylobacter jejuni*, consisting of only 984 amino acids. This variant recognizes the PAM 5′-NNNVRYM (where V = A, C, or G; R = A or G; Y = C or T) and has been successfully employed for *in vivo* genome editing (70, 71). Further efforts to explore and distinguish Cas9 orthologues led to the identification of CasX, a protein composed of 980 amino acids. However, detailed functional characterization of CasX remains limited (72).

Cas 12a

Cas12a, also known as Cpf1, is a class II RNA-guided endonuclease that has been modified for precise genetic modifications in human cells (73). Cas12a, functioning within the type V CRISPER framework, Cas12a introduces precise incision at specific DNA target sites, resulting in a 5′ overhang. Unlike Cas9, Cas12a does not require a transactivating crRNA, as seen in Fig. 2. Cas12a produces clean DNA ends, making it advantageous for applications that demand precise DNA sequence alignment, in contrast to the blunt or imprecise ends often associated with Cas9.

Moreover, Cas12a possesses the unique ability to process its own crRNA arrays, enabling the autonomous generation of individual crRNAs from a single transcript. This feature facilitates multiplexed genome editing using a single customized crRNA array, thereby simplifying the delivery of multiple guide RNAs (34). PAM, a specific DNA sequence essential for target recognition, is located at the 5′ end of the target site for Cas12a. The Cas12a enzymes derived from *Acidaminococcus spp*. (AsCas12a) and *Lachnospiraceae spp*. (LbCas12a) were the first identified variants shown to function efficiently in human cells.

To further advance genome editing capabilities, an enhanced variant of AsCas12a, termed enAsCas12a, has been developed (72). Recent innovations have led to the creation of additional AsCas12a variants with expanded PAM recognition capabilities. These variants are capable of identifying PAM sequences such as 5′-VTTV, 5′-TTTT, 5′-TTCN and 5′-TATV (35), as well as 5′-TYCV and 5′-TATV (36). The distinct structural and functional features of Cas12a, along with its unique cleavage mechanism, enhance its efficiency as a genome editing tool and significantly broaden the potential applications of CRISPR technology.

Mechanism of CRISPR Genome Editing

Gene editing is made possible by CRISPR technologies that cause double strand breaks (DSBs) at certain regions of the genome, which are subsequently repaired by intrinsic cellular mechanisms. Researchers rapidly adopted the RNA-guided, DNA-targeting CRISPR/Cas system due to its user-friendliness and lower cost compared to earlier genome editing techniques such zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). The CRISPR/Cas system originates from the bacterial immune defense mechanism that protects the host against invading nucleic acids such as phages and viruses (74). According to the taxonomy, there are three kinds of CRISPR/Cas methods, each consisting of multiple subgroups. Among these, the type II system is the predominant method used for gene editing and includes three essential components: transactivating crRNA (tracrRNA), CRISPR RNA (crRNA) and the endonuclease (Cas9) (9). The combination of tracrRNA and crRNA form a duplex structure known as guide RNA (gRNA). This gRNA may be substituted with a chimeric single guide RNA (sgRNA) (9). This sgRNA has a 20 base-pair (bp) sequence complementary to the target DNA site and = must be followed by a PAM sequences, which stands for "protospacer-adjacent motif". The presence of PAM sequences is necessary for compatibility with the Cas9 Protein (76).

Within a cell, the sgRNA and Cas9 nuclease together form a ribonucleoprotein (RNP) complex. The sgRNA directs this complex to a specific DNA sequence through Watson-Crick base pairing, where Cas9 induces a double-strand break. The DNA is cleaved three nucleotides upstream of the PAM, resulting in blunt ends. This cleavage is mediated by the RuvC and HNH nuclease domains at the Cas9 active site, which act on the negative (-) and positive (+) strands, respectively (57, 58).

Two cellular processes that repair double-strand breaks (DSBs): homology-directed repair (HDR) and non-homologous end joining (NHEJ). The choice of repair mechanism depends on the overall cellular context and the availability of a homologous repair template (77-79). HDR enables precise genome editing by using a donor DNA template to accurately repair the break site. In contrast, NHEJ, which often results in insertions or deletions (indels) at the cleavage site, is an error-prone mechanism that can be exploited to generate targeted gene knockouts (KOs) via frameshift mutations (Fig. 3)

The CRISPR/Cas9 system allows for simultaneous editing at multiple genomic sites by targeting different loci with sgRNAs. Utilizing two sgRNAs can induce small deletions within the same cell, occasionally leading to complex genomic rearrangements. In certain suppressive conditions, such deletions may even impact entire chromosomes. Modifications to the CRISPR/Cas9 system, including the incorporation of various interacting molecules, have enhanced its specificity and broadened its applicability in genome editing. However, numerous studies have reported off-target effects associated with CRISPR technology, underscoring the need for improved DNA targeting specificity. Various strategies have been developed to minimize unintended modifications and there remains a critical need to further refine CRISPR/Cas9 for more accurate and efficient gene editing applications.

Applications of CRISPR-Cas system

CRISPR-Cas sytem in drug discovery

The process of drug research and discovery is intricate, including the identification and subsequent commercialization of novel medicines. This field of study is fundamentally based on biological hypotheses, aiming to explore the effects of a single treatment on the progression or modification of a specific disease. The primary objective is to pinpoint the exact gene responsible for the disease in order to develop a targeted therapy through genome engineering. However, this process is often time-consuming.

Our research is primarily focused on engineering the CRISPR/Cas system. This technology contributes significantly to drug development by targeting specific molecular pathways involved in disease activation or inhibition, thereby aiding in

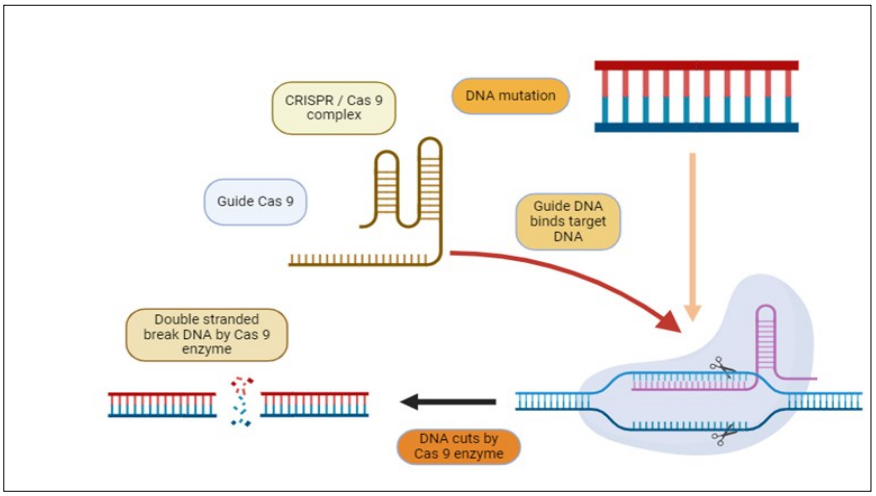

Fig. 3. Genome editing or frameshift mutations of targeted gene knockouts.

disease prevention. Under selective pharmacological conditions, cells capable of surviving are identified by the presence of genes under positive selection.

For example, cells may first undergo treatment with CRISPR sequences and thereafter be subjected to an anti-cancer therapy. Only those cells that develop drug resistance are collected for further analysis of their guide RNA (gRNA) sequences. These gRNAs are then used to identify potential genes associated with drug resistance.

Negative selection, on the other hand, involves the identification of slow-growing or dead cells under certain circumstance. This approach is beneficial for identifying certain genes that are crucial for survival and have the potential to be viable candidates for targeted pharmaceuticals.

CRISPR/Cas9 system in drug resistance

Finding genes linked to drug resistance is a crucial component of drug development and a major use of CRISPR/Cas9 technology. Mutagenesis, which occurs during cell division, is used to evaluate the effectiveness of anticancer treatments in order to find drug-resistant agents. The study illustrates that only cells with a mutation affecting drug application activity may survive. CRISPR/Cas9 technology enables the precise identification of gene deletions that lead to drug resistance. CRISPR/Cas9 technology enables the precise identification of gene deletions that lead to drug resistance. When a population of cells is exposed to a library of CRISPR/Cas9 guide RNAs (gRNAs) targeting various genes for knockout, those cells that acquire resistance to a specific drug can be isolated and studied.

The emergence of drug-resistant in these cells indicates that specific gene disruptions contribute to reduced drug sensitivity. The drug-resistance–associated genes identified through this approach can subsequently be targeted using alternative therapeutic strategies to circumvent resistance. For instances, disruption of the HPRT1 gene via CRISPR/Cas9 editing can render cells resistant to 6-thioguannie, which is commonly used in standard anti-cancer treatment. Similarly, modification of the XPO1 gene using the CRISPR/Cas9 system can induce resistance to Selinexor (80).

Future aspects of CRISPR-Cas sytem

CRISPR-Cas is a cutting-edge technology that is increasingly being used for the purpose of editing genomes, as well as for therapeutic applications. It has shown promising results in clinical settings. This method is efficient and ensures secure delivery to the intended location. The taxonomy of CRISPR-Cas systems provides a fundamental framework for genome editing and evolutionary research. Nonetheless, a novel form of the CRISPR-Cas system continues to undergo refinement and enhancement via the development of multiple variations. Further, optimization remains essential to advance the efficacy and reliability of this gene editing method.

Ongoing advancements in this technology are expected to expand its therapeutic applications across several domains. The use of CRISPR/Cas9 screens will facilitate in identifying new therapeutic targets, thereby requiring extensive in-depth investigation to get a comprehensive understanding of the underlying biological processes. Furthermore, it is crucial to enhance the in vivo use of CRISPR/Cas9 by advancing viral and non-viral delivery methods. This will pave the way for the future therapeutic usage of CRISPR, improving its effectiveness and potential in medical applications.

Conclusion

The CRISPR/Cas9 technology, which was discovered in 2012 and inspired by the natural defense mechanisms of bacteria against viruses, is a ground breaking tool for accurately modifying genomes. The Cas9 protein, guided by a specific RNA sequence, enables targeted cleavage of DNA at designated sites, facilitating accurate genetic modifications. The applications of this technology are extensive, encompassing fields such as cancer research, where it aids in the development of targeted therapies and gene therapy, which focuses on correcting genetic mutations. Beyond medicine, CRISPR/Cas9 holds significant potential in agriculture, environmental conservation and synthetic biology. However, the rapid advancement of this technology necessitates careful consideration of ethical and safety concerns. Its responsible implementation requires collaboration among scientists, regulatory bodies and bioethicists to ensure safe and ethical usage while minimizing associated risks. This collaborative approach seeks to

maximize the benefits of CRISPR/Cas9 in both scientific and clinical domains.

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Authors' contributions

KDR and KKC drafted this manuscript. KKC helped in conception and design of this manuscript. KKC, DD, TS and ST participated in literature search and synthesis. SS, SYM, AKP and AKS edited and revised this manuscript. SSB, NK, DS participated in figures and tables. AKP, AKS, SSB and NK participated in referencing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare

Ethical issues: None

References

1. Hossain MA. CRISPR-Cas9: A fascinating journey from bacterial immune system to human gene editing. Prog Mol Biol Transl Sci. 2021;178:63–83. https://doi.org/10.1016/bs.pmbts.2021.01.001
2. Hussen BM. CRISPR/Cas9 gene editing: A novel strategy for fighting drug resistance in respiratory disorders. Cell Commun and Sign. 2024;22(329):1–21. https://doi.org/10.1186/s12964-024-01713-8
3. Heidenreich M, Zhang F. Applications of CRISPRCas systems in neuroscience. Nat Rev Neurosci. 2016;17:36–44. https://doi.org/10.1038/nrn.2015.2
4. Lucas D, O'Leary HA, Ebert BL, Cowan CA, Tremblay CS. Utility of CRISPR/Cas9 systems in haematology research. Exp Hematol. 2017;54:1–3. https://doi.org/10.1016/j.exphem.2017.06.006
5. Biagioni A, Laurenzana A, Margheri F, Chilla A, Fibbi G, Rosso MD. Delivery systems of CRISPR/Cas9- based cancer gene therapy. J Biol Eng. 2018;12(33):1–9. https://doi.org/10.1186/s13036-018-0127-2
6. Yin H, Xue W, Anderson DG. CRISPR-Cas: A tool for cancer research and therapeutics. Nat Rev Clin Oncol. 2019;16(5):281–95. https://doi.org/10.1038/s41571-019-0166-8
7. Liu J, Zhou G, Zhang L, Zhao Q. Building potent chimeric antigen receptor T cells with CRISPR genome editing. Front Immunol. 2019:10:456. https://doi.org/10.3389/fimmu.2019.00456
8. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, et al. Multiplex genome engineering using CRISPR/Cas systems. Science 2013;339 (6121),819–23. https://doi.org/10.1126/science.1231143
9. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNAv endonuclease in adaptive bacterial immunity. Science. 2012;337(6096):816–21. https://doi.org/10.1126/science.1225829
10. Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, et al. RNA-guided human genome engineering via Cas9. Science. 2013;339 (6121):823–26. https://doi.org/10.1126/science.1232033
11. Jinek M, Jiang F, Taylor DW, Sternberg SH, Kaya E, Ma E, et al. Structures of Cas9 endonucleases reveal RNA-mediated conformational activation. Science. 2014;343(6176):1247997. https://doi.org/10.1126/science.1247997
12. Sternberg SH, Redding S, Jinek M, Greene EC, Doudna JA. DNA interrogation by the CRISPR RNA-guided endonuclease Cas9. Nature. 2014;507(7490):62–67. https://doi.org/10.1038/nature13011
13. Zuo Z, Liu J. Cas9-catalysed DNA cleavage generates staggered ends: evidence from molecular dynamics simulations. Sci Rep. 2016;6(37584):1–9. https://doi.org/10.1038/srep37584
14. Lemos BR, Kaplan AC, Bae JE, Ferrazzoli AE, Kuo J, Anand RP, et al. CRISPR/Cas9 cleavages in budding yeast reveal templated insertions and strand-specific insertion/ deletion profiles. Proc Natl Acad Sci U S A. 2018;115(9): E2040–47. https://doi.org/10.1073/pnas.1716855115
15. Kim S, Kim D, Cho SW, Kim J, Kim JS. Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoproteins. Genome Res. 2014;24(6):1012–19. https://doi.org/10.1101/gr.171322.113
16. Lin S, Staahl BT, Alla RK, Doudna JA. Enhanced homology-directed human genome engineering by controlled timing of CRISPR/Cas9 delivery. eLife. 2014;3:e04766. https://doi.org/10.7554/eLife.04766
17. Maruyama T, Dougan SK, Truttmann MC, Bilate AM, Ingram JR, Ploegh HL. Increasing the efficiency of precise genome editing with CRISPR-Cas9 by inhibition of nonhomologous end joining. Nat Biotechnol. 2015;33(5):538–42. https://doi.org/10.1038/nbt.3190
18. Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, et al. CRISPR provides acquired resistance against viruses in prokaryotes. Science. 2007;315:1709–12. https://doi.org/10.1126/science.1138140
19. Makarova KS, Wolf YI, Alkhnbashi OS, Costa F, Shah SA, Saunders SJ, et al. An updated evolutionary classification of CRISPR-Cas systems. Nat Rev Microbiol. 2015;13(11):722–36. https://doi.org/10.1038/nrmicro3569
20. Shmakov S, Smargon A, Scott D, Cox D, Pyzocha N, Yan W, et al. Diversity and evolution of class 2 CRISPR-Cas systems. Nat Rev Microbiol. 2017;15(3):169–82. https://doi.org/10.1038/nrmicro.2016.184
21. Koonin EV, Makarova KS, Zhang F. Diversity, classification and evolution of CRISPR-Cas systems. Curr Opin Microbiol. 2017;37:67–78. https://doi.org/10.1016/j.mib.2017.05.008
22. Oliver AP, Gersbach CA. The next generation of CRISPR–Cas technologies and applications. Nat Rev Mol Cell Biol. 2020;20 (8):490–507. https://doi.org/10.1038/s41580-019-0131-5
23. Yoshimi K, Mashimo K. Genome editing technology and applications with the type I CRISPR system. Gen Genom Edit Cell Biol. 2022;3-4:100013. https://doi.org/10.1016/j.ggedit.2022.100013
24. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell. 2014;157:1262–78. https://doi.org/10.1016/j.cell.2014.05.010
25. Ishino Y, Shinagawa H, Makino K, Amemura M, Nakatura A. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isoenzyme conversion in *Escherichia coli* and identification of the gene product. J Bacteriol. 1987;169:5429–33. https://doi.org/10.1128/jb.169.12.5429-5433.1987
26. Groenen PM, Bunschoten AE, Soolingen DV, Embden JDV. Nature of DNA polymorphism in the direct repeat cluster of *Mycobacterium tuberculosis*; application for strain differentiation by a novel typing method. Mol Microbiol. 1993;10 (5):1057–65. https://doi.org/10.1111/j.1365-2958.1993.tb00976.x
27. Hoe N, Nakashima K, Grigsby D, Pan X, Dou SJ, Naidich S, et al. Rapid molecular genetic subtyping of serotype M1 group A Streptococcus strains. Emerg Infect Dis. 1999;5(2):254–63. https://doi.org/10.3201/eid0502.990210
28. Mojica FJM, Juez G, Rodriguez-Valera F. Transcription at different

salinities of Haloferax mediterranei sequences adjacent to partially modified PstI sites. Mol Microbiol. 1993;9,613–21. https://doi.org/10.1111/j.1365-2958.1993.tb01721.x

29. Mojica FJM, Díez-Villaseñor C, García-Martínez J, Soria E. Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. J Mol. Evol., 2005;60:174–82. https://doi.org/10.1007/s00239-004-0046-3.

30. Bolotin A, Quinquis B, Sorokin A, Dusko Ehrlich S. Clustered regularly interspaced short palindrome repeats (CRISPRS) have spacers of extrachromosomal origin. Microbiology. 2005;151:2551–61. https://doi.org/10.1099/mic.0.28048-0

31. Pourcel C, Salvignol G, Vergnaud G. CRISPR elements in Yersinia pestis acquire new repeats by preferential uptake of bacteriophage DNA and provide additional tools for evolutionary studies. Microbiology. 2005;151:653–63. https://doi.org/10.1099/mic.0.27437-0

32. Popkov V A, Zorova LD, Korvigo IO, Silachev DN, Jankauskas SS, et al. Do mitochondria have an immune system? Biochemistry (Moscow). 2016;81:1229–36. https://doi.org/10.1134/S0006297916100217

33. Isaev AB, Musharova OS, Severinov KV. Microbial arsenal of antiviral defences. Part I, Biochemistry (Moscow). 2021; 86:319–37. https://doi.org/10.1134/S0006297921030081

34. Marraffini LA, Sontheimer EJ. CRISPR interference limits horizontal gene transfer in *staphylococci* by targeting DNA. Science. 2008;322:1843–45. https://doi.org/10.1126/science.1165771

35. Shmakov S, Abudayyeh OO, Makarova KS, Wolf YI, Gootenberg JS, et al. Discovery and functional characterization of diverse class 2 CRISPR–Cas systems. Mol Cell. 2015;60:385–97. https://doi.org/10.1016/j.molcel.2015.10.008

36. Shmakov S, Smargon A, Scott D, Cox D, Pyzocha NV, et al. Diversity and evolution of class 2 CRISPR–Cas systems. Nat Rev Microbiol. 2017;15:169–82. https://doi.org/10.1038/nrmicro.2016.184

37. Abudayyeh OO, Gootenberg JS, Konermann S, Joung J, Slaymaker IM, et al. C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. Science 2016;353:aaf5573. https://doi.org/10.1126/science.aaf5573

38. Abudayyeh OO, Gootenberg JS, Essletzbichler P, Han S, Joung J, Belanto JJ, et al. RNA targeting with CRISPR–Cas13. Nature. 2017;550:280–84. https://doi.org/10.1038/nature24049

39. Liu Z, Dong H, Cui Y, Cong L, Zhang D. Application of different types of CRISPR/Cas-based systems in bacteria. Microbial Cell Factories. 2020;19(172):1–14. https://doi.org/10.1186/s12934-020-01431-z

40. Nishimasu H, Ran FA, Hsu PD, Konermann S, Shehata S, Dohmae N. Crystal structure of Cas9 in complex with guide RNA and target DNA. Cell. 2017;176(12):139–48. https://doi.org/10.1016/j.cell.2014.02.001

41. Richter C, Gristwood T, Clulow JS, Fineran PC. *In vivo* protein interactions and complex formation in the *Pectobacterium atrosepticum* subtype I-F CRISPR/ Cas system. PLoS ONE. 2012;7(12):e49549. https://doi.org/10.1371/journal.pone.0049549

42. Deltcheva E, Chylinski K, Sharma CM, Gonzales K, Chao Y, Pirzada ZA, et al. CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. Nature 2011;471(7340):602–07. https://doi.org/10.1038/nature09886

43. Zhang Y, Heidrich N, Ampattu BJ, Gunderson CW, Seifert HS, Schoen C, et al. Processing-independent CRISPR RNAs limit natural transformation in *Neisseria meningitidis*. Mol Cell. 2013;50(4):488–503. https://doi.org/10.1016/j.molcel.2013.05.001

44. Wiedenheft B, Dujin EV, Bultema JB, Waghmare SP, Zhou K, Barendregt A, et al. RNA-guided complex from a bacterial immune system enhances target recognition through seed sequence interactions. Proc Natl Acad Sci USA. 2011;108(25):10092–97. https://doi.org/10.1073/pnas.1102716108

45. Brouns SJ, Jore MM, Lundgren M, Westra, ER, Slijkhuis RJ, Snijders A P, et al. Small CRISPR RNAs guide antiviral defence in prokaryotes. Sci. 2008:321,960–64. https://doi.org/10.1126/science.1159689

46. Gao L, Cox DBT, Yan WX, Manteiga JC, Schneider MW, Yamano T, et al. Engineered Cpf1 variants with altered PAM specificities. Nat Biotechnol. 2017;35(8):789–92. https://doi.org/10.1038/nbt.3900.

47. Jore MM, Lundgren M, Duijn EV, Bultema JB, Westra ER, Waghmare SP, et al. Structural basis for CRISPR RNA-guided DNA recognition by Cascade. Nat Struct Mol Biol. 2011;18(5):529–36. https://doi.org/10.1038/nsmb.2019

48. Westra ER, Erp PBGV, Kunne T, Wong SP, Staals RHJ, Seegers CLC, et al. CRISPR immunity relies on the consecutive binding and degradation of negatively supercoiled invader DNA by Cascade and Cas3. Mol Cell. 2012;46(5):595–605. https://doi.org/10.1016/j.molcel.2012.03.018

49. Hochstrasser ML, Taylor DW, Bhat P, Guegler CK, Stenberg SH, Nogales E, et al. CasA mediates Cas3-catalysed target degradation during CRISPR RNA-guided interference. Proc Natl Acad Sci USA. 2014;111(188):6618–23. https://doi.org/10.1073/pnas.1405079111

50. Brendel J, Stoll B, Lange SJ, Sharma K, Lenz C, Stachler AE, et al. A complex of Cas proteins 5, 6 and 7 is required for the biogenesis and stability of clustered regularly interspaced short palindromic repeats (CRISPR)-derived RNAs (crRNAs) in Haloferax volcanii. J Biol Chem. 2014;289(10):7164–77. https://doi.org/10.1074/jbc.M113.508184

51. Sinkunas T, Gasiunas G, Fremaux C, Barrangou R, Horvath P, Siksnys V. Cas3 is a single-stranded DNA nuclease and ATP-dependent helicase in the CRISPR/Cas immune system. EMBO J. 2011;30(7):1335–42. https://doi.org/10.1038/emboj.2011.41

52. Jackson RN, Lavin M, Carter J, Wiedenheft B. Fitting CRISPR-associated Cas3 into the helicase family tree. Curr Opin Struct Biol. 2014;24:106–14. https://doi.org/10.1016/j.sbi.2014.01.001

53. Gomaa AA, Klumpe HE, Luo ML, Selle K, Barrangou R, Beisel CL. Programmable removal of bacterial strains by use of genome-targeting CRISPR-Cas systems. mBio. 2014;5(1):e00928-00913. https://doi.org/10.1128/mBio.00928-13

54. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012;337(6096):816–21. https://doi.org/10.1126/science.1225829

55. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, et al. Multiplex genome engineering using CRISPR/Cas systems. Science 2013;339(6021):819–23. https://doi.org/10.1126/science.1231143

56. Mali P, Yang L, Esvelt KM, Arch J, Guell M, DiCarlo JE, et al. RNA-guided human genome engineering via Cas9. Science. 2013;339(6121):823–26. https://doi.org/10.1126/science.1232033

57. Mojica FJ, Diez-Villasenor C, Garcia-Martinez J, Almendros C. Short motif sequences determine the targets of the prokaryotic CRISPR defence system. Microbiology. 2009;155(Pt3):733–40. https://doi.org/10.1099/mic.0.023960-0

58. Gasiunas G, Barrangou R, Horvath P, Siksnys V. Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proc Natl Acad Sci USA. 2012;109(39):E2579–86. https://doi.org/10.1073/pnas.1208507109

59. Kleinstiver BP, Prew MS, Tsai SQ, Topkar VV, Nguyen NT, Zheng Z, et al. Engineered CRISPR-Cas9 nucleases with altered PAM specificities. Nature. 2015;523(7561):481–85. https://doi.org/10.1038/nature14592

60. Hu JH, Miller SM, Geurts MH, Tang W, Chen L, Sun N, et al. Evolved Cas9 variants with broad PAM compatibility and high DNA specificity. Nature. 2018;556(7699):57–63. https://doi.org/10.1038/nature26155

61. Slaymaker IM, Gao L, Zetsche B, Scott DA, Yan WX, Zhang F. Rationally engineered Cas9 nucleases with improved specificity. Science. 2016;351(6268):84–88. https://doi.org/10.1126/science.aad5227

62. Kleinstiver BP, Pattanayak V, Prew MS, Tsai SQ, Nguyen NT, Zheng Z, et al. High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. Nature. 2016;529(7587):490–95. https://doi.org/10.1038/nature16526

63. Chen JS, Dagdas YS, Kleinstiver BP, Welch MM, Sousa AA, Harrington LB, et al. Enhanced proofreading governs CRISPR-Cas9 targeting accuracy. Nature. 2017;550(7676):407–10. https://doi.org/10.1038/nature24268

64. Casini A, Olivieri M, Petris G, Montagna C, Reginata G, Maule G, et al. A highly specific SpCas9 variant is identified by *in vivo* screening in yeast. Nat Biotechnol. 2018;36(3):265–71. https://doi.org/10.1038/nbt.4066

65. Vakulskas CA, Dever DP, Rettig GR, Turk R, Jacobi AM, Collingwood MA, et al. A high-fidelity Cas9 mutant delivered as a ribonucleoprotein complex enables efficient gene editing in human hematopoietic stem and progenitor cells. Nat Med. 2018;24(8):1216–24. https://doi.org/10.1038/s41591-018-0137-0

66. Kocak DD, Josephs EA, Bhandarkar V, Adkar SS, Kwon JB, Gersbach CA. Increasing the specificity of CRISPR systems with engineered RNA secondary structures. Nat Biotechnol. 2019;37(6):657–66. https://doi.org/10.1038/s41587-019-0095-1

67. Deveau H, Barrangou R, Garmeau JE, Labonte J, Fremaux C, Boyaval P, et al. Phage response to CRISPR-encoded resistance in *Streptococcus thermophilus*. J Bacteriol. 2008;190(4):1390–400. https://doi.org/10.1128/JB.01412-07

68. Hou Z, Zhang Y, Propson NE, Howden SE, Chu LF, Sontheimer EJ, et al. Efficient genome engineering in human pluripotent stem cells using Cas9 from *Neisseria meningitidis*. Proc Natl Acad Sci USA. 2013;110(39):15644–49. https://doi.org/10.1073/pnas.1313587110

69. Ran FA, Cong L, Yan WX, Scott DA, Gootenberg JS, Kriz AJ, et al. *In vivo* genome editing using Staphylococcus aureus Cas9. Nature. 2015;520(7546):186–91. https://doi.org/10.1038/nature14299

70. Yamada M, Watanabe Y, Gootenberg JS, Hirano H, Ran FA, Nakane T, et al. Crystal structure of the minimal Cas9 from Campylobacter jejuni reveals the molecular diversity in the CRISPR-Cas9 systems. Mol Cell. 2017;65(6):1109–21. https://doi.org/10.1016/j.molcel.2017.02.007

71. Kim E, Koo T, Park SW, Kim D, Kim K, Cho HY, et al. In vivo genome editing with a small Cas9 orthologue derived from Campylobacter jejuni. Nat Commun. 2017;8(14500):1–12. https://doi.org/10.1038/ncomms14500

72. Burstein D, Harrington LB, Strutt SC, Probst AJ, Anantharaman K, Thomas BC, et al. New CRISPR-Cas systems from uncultivated microbes. Nature. 2017;542(7640):237–41. https://doi.org/10.1038/nature21059

73. Zetsche B, Heidenreich M, Mohanraju P, Fedorova I, Kneppers J, DeGennaro EM, et al. Multiplex gene editing by CRISPR-Cpf1 using a single crRNA array. Nat Biotechnol. 2017;35(1):31–34. https://doi.org/10.1038/nbt.3737

74. Kleinstiver BP, Sousa AA, Walton RT, Tak YE, Hsu JY, Clement K, et al. Engineered CRISPR–Cas12a variants with increased activities and improved targeting ranges for gene, epigenetic and base editing. Nat Biotechnol. 2019;37(3):276–82. https://doi.org/10.1038/s41587-018-0011-0

75. Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, et al. CRISPR provides acquired resistance against viruses in prokaryotes. Science. 2007;315(5819):1709–12. https://doi.org/10.1126/science.1138140

76. Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. Genome engineering using the CRISPR-Cas9 system. Nat Protoc. 2013;8(11):2281–308. https://doi.org/10.1038/nprot.2013.143

77. Liang F, Han M, Romanienko PJ, Jasin M. Homology-directed repair is a major double-strand break repair pathway in mammalian cells. Proceedings of the National Academy of Sciences. 1998;95(9):5172–77. https://doi.org/10.1073/pnas.95.9.5172

78. Bibikova M, Carroll D, Segal DJ, Trautman JK, Smith J, Kim YG, et al. Stimulation of homologous recombination through targeted cleavage by chimeric nucleases. Molecular and cellular biology. 2001;21(1):289–97. https://doi.org/10.1128/MCB.21.1.289-297.2001

79. Fishman-Lobell J, Rudin N, Haber JE. Two alternative pathways of double-strand break repair are kinetically separable and independently modulated. Molecular and cellular biology. 1992;12(3):1292–303. https://doi.org/10.1128/mcb.12.3.1292-1303.1992

80. Smurnyy Y, Cai M, Wu H, McWhinnie E, Tallarico JA, Yang Y, et al. DNA sequencing and CRISPR-Cas9 gene editing for target validation in mammalian cells. Nat Chem Biol. 2014;10(8):623–25. https://doi.org/10.1038/nchembio.1550

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