# Gene Editing Technology: Advances and Challenges Postprint

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## Abstract

Gene editing refers to the deletion, replacement, insertion, and other manipulations of target genes to acquire novel functions or phenotypes, and even to create new species. As a crucial and rapidly advancing research domain in life sciences, the development and application of gene editing technologies have propelled genetic modification of organisms to unprecedented depths and scales, making significant contributions to the functional dissection of specific genes. This article provides an overview of current gene editing technologies, as well as the challenges and opportunities in the field of gene editing in China, aiming to enhance comprehensive understanding of this technological framework and facilitate the identification of new breakthrough avenues in this domain.

## Full Text

## Preamble

Gene editing refers to the manipulation of target genes through deletion, replacement, insertion, and other operations to obtain new functions or phenotypes, and even create new species. As a rapidly developing field in life sciences, gene editing technologies have enabled genetic modification of organisms to reach unprecedented depth and breadth, making significant contributions to the functional analysis of specific genes. This article provides an overview of current gene editing technologies, as well as the challenges and opportunities facing China in this field, aiming to enhance overall understanding of these technological systems and help identify new breakthrough points.

**Keywords:** gene editing, recombinant nuclease, CRISPR/Cas9, DNA-mediated gene editing
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The rapid development of life sciences has ushered us from the "reading" stage of genetic information into the post-genomic era, where "rewriting" and even "de novo design" of genomes are gradually becoming reality. Synthetic biology, aimed at designing and creating new life forms, has developed rapidly in this context and demonstrated tremendous application potential in medicine, manufacturing, energy, and other fields. De novo genome synthesis and large-scale modification of natural genomes belong to the fields of synthetic genomics and gene editing, respectively, both representing hot topics in current synthetic biology research. Synthetic genomics involves the de novo design, construction, and functional characterization of genomes, representing a bottom-up research strategy in biology; whereas gene editing focuses on rewriting genetic information through molecular operations such as deletion, replacement, and insertion on existing genomes, representing a top-down biological research strategy. The organic integration of these two approaches will greatly drive innovation in biomanufacturing, disease treatment, and other fields, while also providing powerful technical means for functional genomics research in the post-genomic era. The de novo design and synthesis of new life systems require not only technologies for genome sequence synthesis, assembly, and transfer, but also highly efficient, low-off-target editing technologies to enable large-scale genomic modifications. Through continuous exploration, gene editing technologies have evolved from initially relying on naturally occurring homologous recombination in cells to targeted cleavage at virtually any site, with their simplicity and efficiency greatly advancing the development of species genetic modification. Gene editing can provide means for further modification of synthetic life and offer more possibilities for creating new species.

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## Abstract

Gene editing includes genetic manipulations like deletion, replacement, insertion, etc., which aims to obtain new gene functions, phenotypes, and even new species. As a burgeoning field of life science, gene editing has brought genetic manipulation into an unprecedented grand state. Here, we summarize the developments of gene editing technologies, and the challenges and opportunities we currently face, aiming to deepen comprehension of these technical systems and help identify breakthrough points in this field.

**Keywords:** gene editing, recombinant nuclease, CRISPR/Cas9, DNA-mediated gene editing
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## Gene Editing Principles

Gene editing technology is an application of DNA double-strand break (DSB) phenomena and their repair mechanisms in organisms. As a common molecular biology event, DSBs occur daily in actively dividing mammalian cells[1-4]. Following DSB occurrence, cells can repair them through multiple pathways, including classical non-homologous end joining (NHEJ), alternative end joining (a-EJ), single-strand annealing (SSA), and homologous recombination (HR)[4]. HR enables precise and error-free repair but requires the presence of a homologous template[5-7]; NHEJ directly ligates two DNA ends with largely no homology[7], during which process both ends typically undergo deletion of several nucleotides, representing an imprecise repair mechanism[8]. As auxiliary repair mechanisms, both a-EJ and SSA require more extensive resection of single-stranded ends[4], which also leads to loss of genetic information. Based on the principle of DNA break repair, if a specific homologous recombination template is artificially provided in cells, and DSBs occur naturally or are induced at target DNA sites, triggering HR repair, specific DNA sequences can be deleted or exogenous genes inserted. Without providing a homologous template, the imprecise repair mechanisms of NHEJ, a-EJ, and SSA can be utilized to achieve gene mutation and knockout. Traditional gene editing relied on naturally occurring DSBs in cells to achieve targeted integration[9], accomplishing gene knockout, replacement, and other purposes. However, in eukaryotic cells, the probability of achieving targeted gene editing through spontaneous DSBs is typically as low as one in a million[10,11]. Artificial methods such as chemical inducers or radiation treatment, or transposon technologies, can also induce mutations, but these mutations are random and require extensive subsequent screening to obtain the desired genotype. Site-specific gene editing technology represents the preferred strategy for gene function research and species-directed modification.

### Recombinant Nuclease-Mediated Gene Editing Technology

The development of artificial nuclease technology has enabled the induction of DSBs at specific sites, with zinc finger nuclease (ZFN) technology representing a milestone breakthrough, also known as the first-generation gene editing technology. ZFN consists of a zinc finger protein (ZFP) and the nuclease domain of the FokI endonuclease, with the former responsible for DNA recognition and the latter for DNA cleavage. ZFP is a naturally occurring protein structure composed of zinc finger (ZF) motifs, each recognizing a specific 3-base pair sequence[11] (Figure 1a [Figure 1: see original paper]). Therefore, the recognition specificity of ZFN can be adjusted by varying the number of tandem ZFs. FokI is connected to ZFP via its N-terminus, and since FokI functions as a dimer,

ZFNs must be designed in pairs[12]. As a novel gene editing tool, ZFN has been used for gene editing in various species since 2001[11,13].

### 2.2 TALENs

ZFN technology ushered gene editing into an era no longer solely dependent on naturally occurring DSBs, but it has significant limitations, including high costs and difficulty in achieving multiplexed editing[11]. The discovery of TALE (transcription activator-like effector) motifs gave rise to the second-generation gene editing technology—TALENs (TALE nucleases). TALENs have a similar architecture to ZFNs, with TALE motifs tandemly arrayed to form the DNA recognition module that determines targeting specificity, connected to the FokI domain. Unlike ZF motifs, a single TALE motif recognizes one base pair (Figure 1b), establishing a one-to-one correspondence between tandem TALE motifs and the recognized base pairs[11]. Studies have found that TALENs achieve the same cleavage efficiency as ZFNs for identical targets, but with typically lower toxicity and easier construction[11]. However, TALENs are considerably larger than ZFNs and contain more repetitive sequences, making their coding genes more difficult to assemble in *Escherichia coli*[11].

## 3 RNA-Guided Gene Editing Technology

### 3.1 CRISPR/Cas9

The CRISPR/Cas system originally evolved in bacteria and archaea as an adaptive immune system to defend against foreign viruses and plasmid DNA. The Type II CRISPR/Cas system relies on the integration of foreign DNA fragments into clustered regularly interspaced short palindromic repeat (CRISPR) loci. After transcription and processing, short CRISPR RNAs (crRNAs) are produced, which anneal with trans-activating crRNA (tracrRNA) to guide the Cas9 (CRISPR-associated protein 9) protein in mediating sequence-specific degradation of foreign DNA[11,14-16]. Jinek et al.[17] discovered that the crRNA and tracrRNA required for Cas9-mediated targeted cleavage could be fused into a single guide RNA (sgRNA) (Figure 1c). Subsequently, several research groups reported that the CRISPR/Cas9 system could be used for targeted gene editing in human cells[18-21]. Compared with ZFN and TALEN technologies, CRISPR/Cas9 is much simpler to design and significantly less expensive, achieving comparable or even better targeting efficiency for the same target sites[11].

### 3.2 CRISPR/Cas9 Derivatives

With deepening research into the CRISPR/Cas system, the catalytic mechanism of the Cas9 nuclease has been elucidated[17], and through mutations at specific amino acid sites, Cas9 nickase (Cas9n) with single-strand cleavage activity or completely inactive dead Cas9 (dCas9) can be obtained. These different Cas9 nucleases have given rise to more versatile gene editing systems with broader applicability.

**(1) CRISPR/Cas9-nickase gene editing technology.** The crRNA used in the CRISPR/Cas9 system can tolerate a certain degree of mismatch, leading to off-target effects that limit the application of CRISPR/Cas9 in high-precision editing[22]. To improve the precision of the Cas9 editing system, Ran et al.[22] cleverly utilized the nickase activity of the Cas9 D10A mutant to design a "single site, dual sgRNA targeting" strategy, namely the CRISPR/Cas9-nickase gene editing technology (Figure 2a [Figure 2: see original paper]). Its principle is similar to ZFN and TALEN: two Cas9n/sgRNA complexes simultaneously target one site, each cleaving one DNA strand to achieve a double-strand break, thereby inducing NHEJ or HR repair. Using this strategy, off-target efficiency in cell lines can be reduced by up to nearly four orders of magnitude.

**(2) CRISPR/dCas9-FokI gene editing technology.** Also to address the off-target issue of CRISPR/Cas9 technology, Guilinger et al.[23] adopted a dCas9-based strategy. Theoretically, dCas9/sgRNA can only function as a simple targeting guide without inducing DNA cleavage, similar to the DNA-binding domain in ZFN or TALEN. To achieve DNA cutting, they introduced the cleavage domain of the FokI endonuclease (Figure 2b) and fused it with dCas9 to create a fusion protein fCas9, echoing the design strategy of ZFN and TALEN. In gene editing of human cells, fCas9 exhibits over 140-fold higher specificity than wild-type Cas9. Moreover, at highly similar off-target sites, fCas9 shows at least 4-fold higher specificity than Cas9n. The application of fCas9 will further enrich the Cas9 toolkit and provide more sophisticated gene editing tools.

**(3) CRISPR/dCas9-based single-base editing technology.** Most human genetic diseases are associated with point mutations in genes[24]. If these could be repaired through precise means, new therapeutic strategies might emerge. While site-specific mutations can be achieved through CRISPR/Cas9 when a homologous recombination template is provided, the random insertions or deletions caused by NHEJ repair represent potential risk factors. Cas9 mutants Cas9n and dCas9 lack the ability to cleave double-stranded DNA but can still perform target localization. If proteins/domains capable of catalyzing specific base conversions are available, CRISPR/Cas9n/dCas9-guided single-base editing technologies can be constructed, referencing the design of CRISPR/dCas9-FokI. The Liu research group fused rat-derived cytosine deaminase (APOBEC1) with dCas9 (Figure 2c) and found that it could site-specifically convert C to U, which then leads to C:G to T:A base pair conversion during subsequent DNA replication or repair[24]. Subsequently, the Kondo group from Kobe University in Japan[25] and the Chang Xing group from Shanghai Jiao Tong University in China[26] reported similar findings. To achieve A:T to G:C conversion, the Liu group employed protein evolutionary engineering to modify the tRNA adenosine deaminase (TadA) from *E. coli*. Their seventh-generation adenine base editors (ABEs) can efficiently mediate A:T to G:C base pair conversion[27]. Thus, free conversion between C to T and G to A has been achieved, and it may become possible to accomplish arbitrary conversion among all four bases in the future.

**(4) CRISPR/dCas9-based gene expression regulation technology.** In

addition to direct DNA editing, the CRISPR/Cas system can also function in gene expression regulation[28,29]. For instance, by exploiting the feature that dCas9 lacks endonuclease activity but can still bind DNA, it can directly block the binding of other factors to DNA, thereby affecting gene expression (Figure 2d). If transcriptional repressors or activators are fused with dCas9, targeted gene inhibition and activation can be achieved, providing flexible operational tools for gene function studies.

### 3.3 CRISPR/Cas12a

CRISPR/Cas9 technology is limited by G-rich PAM sequences and cannot target arbitrary sequences. Additionally, the Cas9 protein is relatively large, which can be inconvenient in certain contexts. In fact, nearly all archaea and numerous bacteria employ CRISPR/Cas mechanisms for immune defense[30], encompassing diverse CRISPR/Cas systems. All characterized Class 2 CRISPR/Cas systems belong to Type II CRISPR/Cas systems that utilize Cas9 family nucleases as effectors. However, another Class 2 CRISPR/Cas system exists in *Prevotella* and *Francisella* 1, classified as Type V CRISPR/Cas system[30]. In 2015, Zhang Feng's team reported that Cpf1 (CRISPR from *Prevotella* and *Francisella* 1) (now called "Cas12a" ) in the Type V system is a functional bacterial immune mechanism capable of mediating effective gene editing in human cells[30]. CRISPR/Cas12a possesses advantages not found in CRISPR/Cas9[30], one being that Cas12a requires T-rich PAM sequences, facilitating its application in species with A/T-rich genomes. The Chen Jia group at ShanghaiTech University fused DNA cleavage-inactive dCas12a with rat-derived cytosine deaminase (APOBEC1) and found that, similar to Cas9-based base editors, it could efficiently catalyze C to T conversion in human cells[31]. Since it recognizes T-rich PAM sequences, the Cas12a-based base editing system complements the Cas9-based base editing system, providing more comprehensive technical conditions for related basic research and future clinical applications.

Beyond DNA editing, the Zhang Feng group discovered that C2c2 (now called Cas13a) in the CRISPR protein family can target and cleave RNA[32], and subsequently demonstrated that Cas13a can target and reduce RNA levels in mammalian cells[33]. Leveraging the RNA targeting function of Cas13a, the CRISPR/Cas13a system has been developed as an RNA detector for disease diagnosis[34]. The Zhang Feng team later discovered Cas13b[35], which also possesses RNA targeting and editing functions. Additionally, Cas13c and Cas13d exhibit similar functions[36]. The establishment of RNA editing technology further expands the application scope of CRISPR/Cas gene editing technology.

## 4 DNA-Mediated Gene Editing Tools

Based on the principle of base complementary pairing and primer design, oligonucleotide (Oligo) DNA can be used. Similar to ZFN and TALEN, Oligo DNA can replace their DNA-binding domains, with the key being to find an appropriate connection method between Oligo DNA and the FokI cleavage

domain. Alternatively, one could search for natural proteins similar to Cas9 that inherently possess endonuclease activity while binding Oligo DNA of a certain length as a guide. Due to the greater stability of DNA itself and the low preparation cost of Oligo DNA, gene editing procedures can be simplified. Oligo DNA-guided gene editing tools offer incomparable advantages over other editing tools and warrant intensive research and development. Particularly given that current mainstream gene editing tools are all patented abroad, which is unfavorable for the future market entry of domestic gene editing products, gene editing technologies with independent intellectual property rights urgently need to be developed.

In September 2016, *Genome Biology* published a novel gene editing tool developed by a research team from Nanjing University: structure-guided endonuclease (SGN)[37]. SGN is a typical Oligo DNA-guided gene editing tool, essentially also representing the design and application of recombinant nucleases. Its N-terminus is a flap endonuclease-1 (FEN-1) that recognizes DNA 3 flaps, while its C-terminus is the DNA cleavage domain (Fn1) of the FokI endonuclease. SGN achieves targeted cleavage by recognizing the 3 flap generated when guide DNA (gDNA) binds to a specific target site. Unlike CRISPR/Cas9 and its derivatives, SGN has no PAM restriction and can theoretically target any sequence. Experimental results show that SGN cleavage activity is independent of target sequence, and it can be used for gene editing in zebrafish, though its efficiency still has considerable room for improvement. As an original research achievement by Chinese scientists, SGN undoubtedly offers significant advantages: gDNA is readily obtainable and its dosage can be precisely controlled as needed; the possibility of mismatches can be minimized by adjusting gDNA length, among other benefits.

## Challenges and Opportunities for China' s Gene Editing Technology Development

China has made remarkable progress in gene editing technology in recent years. However, we must recognize that core patents related to gene editing, especially CRISPR/Cas9, are currently held by other countries. This will result in significant profit losses as gene editing technologies and cell products move toward clinical applications and as gene-edited crops enter the market. Developing core technologies with independent intellectual property rights is essential for China to gain ground in this biotechnology revolution, including correcting defects in existing gene editing technologies and, through technological combinations, securing improved and enhanced versions of current gene editing technologies ahead of competitors. Additionally, bioinformatics approaches should be employed to mine potential CRISPR nucleases and DNA-guided nucleases to develop novel gene editing technologies. Moreover, gene editing technologies, particularly CRISPR/Cas9, have been widely used for gene editing across various species, including common model organisms such as nematodes, fruit flies, zebrafish, and mice, as well as large animals like pigs, dogs, and mon-

keys, and major crops such as rice and wheat. Expanding existing gene editing technologies to other organisms also represents a new breakthrough, especially for China' s unique biological resources. This process often involves technical adjustments and improvements that can generate new technologies while providing effective tools for functional genomics research and genetic improvement of target organisms.

Currently, CRISPR/Cas9 and other gene editing technologies have demonstrated tremendous application value, but many issues remain to be resolved regarding editing efficiency, precision, off-target effects, and their progression to clinical applications. Viral vectors can be applied to human gene therapy, but their loading capacity is limited, and the conventional Cas9 protein is too large for convenient use. On one hand, smaller Cas9 proteins can be sought from nature; on the other hand, appropriate truncation can be performed through genetic engineering. Regarding off-target reduction, in addition to the aforementioned CRISPR/Cas9-nickase and CRISPR/dCas9-FokI gene editing technologies, Cas9 protein itself can be engineered through site-directed mutagenesis to enhance its specificity. These endeavors are challenging but also represent opportunities and breakthrough points for our development.

In recent years, Chinese scientists have made substantial progress in the field of gene editing. In addition to the breakthroughs by the Chang Xing and Chen Jia groups in base editing technology mentioned above, the Huang Zhiwei group from Harbin Institute of Technology and the Wang Yanli group from the Institute of Biophysics, Chinese Academy of Sciences, have made outstanding contributions to structural elucidation of Cas/sgRNA complexes, providing important references for understanding gene editing mechanisms. The Gao Caixia group from the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, has achieved significant progress in plant gene editing. The Lai Liangxue group from Guangzhou Institutes of Biomedicine and Health, the Ji Weizhi group from Kunming University of Science and Technology, and the Yang Hui group from the Institute of Neuroscience, Chinese Academy of Sciences, among others, have successfully obtained various disease animal models using gene editing technologies. China has also achieved certain breakthroughs in developing novel gene editing technologies; DNA-guided gene editing technology possesses unique advantages and deserves increased investment to address issues such as low editing efficiency.

Furthermore, as an emerging interdisciplinary field, synthetic biology has demonstrated increasingly broad application potential in biomedicine, energy, new materials, and other areas. Genome synthesis and gene editing involve different operational breadths, depths, and technical systems. However, both essentially aim to obtain organisms with specific functions through genetic modification to serve research and production, and their organic integration represents a natural trend. For instance, gene editing systems can be introduced into synthetic genomes to provide more possibilities for further modification and application of new organisms. SCRaMbLE (synthetic chromosome

rearrangement and modification by loxP-mediated evolution)[38] is a rapid chromosome rearrangement and modification technology that can accelerate organism evolution, essentially representing a non-site-specific, inducible gene editing technology. Biological evolution often requires lengthy historical processes, making it difficult to rapidly obtain species with desired traits. Synthetic biology can introduce specific functional modules through genome design to obtain engineered cells; however, given current cost and technical limitations, although many candidate genome designs may exist, obtaining a large-scale synthetic cell library and screening for optimal designs is not feasible. SCRaMbLE represents a potentially cost-effective solution. The Sc2.0 project plans to insert approximately 5,000 loxPysm sites into the synthetic yeast genome[39]. Recent series of publications from this project[40-46] have demonstrated that the SCRaMbLE system can induce enhanced industrial yeast strains with increased production of target metabolites.

Currently, the first fully artificially synthesized eukaryote—Sc2.0—is nearing completion, and whole-genome synthesis of higher organisms has been put on the agenda[47]. However, synthesizing ultra-large genomes still faces many challenges. Local genome modification of existing species through gene editing essentially represents an upgrade of traditional genetic engineering and is unlikely to achieve comprehensive genome rewriting. At present, the combination of genome synthesis and genome editing represents a highly promising research direction that could address various difficulties in de novo synthesis of ultra-large genomes and provide possibilities for applying synthetic genomics in basic research and species modification of organisms with large genomes. This also represents an opportunity that China can seize in the fields of synthetic biology and gene editing.

## References

1. Martin G M, Smith A C, Ketterer D J, et al. Increased chromosomal aberrations in first metaphases of cells isolated from the kidneys of aged mice. *Isr J Med Sci*, 1985, 21(3): 296-301.
2. Lieber M R, Karanjawala Z E. Ageing, repetitive genomes and DNA damage. *Nat Rev Mol Cell Biol*, 2004, 5(1): 69-75.
3. Lieber M R. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. *Annu Rev Biochem*, 2010, 79: 181-211.
4. Chang H H Y, Pannunzio N R, Adachi N, et al. Non-homologous DNA end joining and alternative pathways to double-strand break repair. *Nat Rev Mol Cell Biol*, 2017, 18(8): 495-506.
5. Paques F, Haber J E. Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. *Microbiol Mol Biol Rev*, 1999, 63(2): 349-404.

6. Sung P, Klein H. Mechanism of homologous recombination: mediators and helicases take on regulatory functions. *Nat Rev Mol Cell Biol*, 2006, 7(10): 739-750.

7. Hartlerode A J, Scully R. Mechanisms of double-strand break repair in somatic mammalian cells. *Biochem J*, 2009, 423(2): 157-168.

8. Lieber M R, Ma Y, Pannicke U, et al. Mechanism and regulation of human non-homologous DNA end-joining. *Nat Rev Mol Cell Biol*, 2003, 4(9): 712-720.

9. Lee A H, Symington L S, Fidock D A. DNA repair mechanisms and their biological roles in the malaria parasite *Plasmodium falciparum*. *Microbiol Mol Biol Rev*, 2014, 78(3): 469-486.

10. Rouet P, Smih F, Jasin M. Introduction of double-strand breaks into the mouse genome by expression of a rare-cutting endonuclease. *Mol Cell Biol*, 1994, 14(12): 8096-8106.

11. Chandrasegaran S, Carroll D. Origins of programmable nucleases for genome engineering. *J Mol Biol*, 2016, 428(5): 963-989.

12. Bibikova M, Golic M, Golic K G, et al. Targeted chromosomal cleavage and mutagenesis in *Drosophila* using zinc-finger nucleases. *Genetics*, 2002, 161(3): 1169-1175.

13. Porteus M H, Baltimore D. Chimeric nucleases stimulate gene targeting in human cells. *Science*, 2003, 300(5620): 763.

14. Mojica F J M, Díez-Villaseñor C, García-Martínez J, et al. Short motif sequences determine the targets of the prokaryotic CRISPR defence system. *Microbiology*, 2009, 155(Pt 3): 733-740.

15. Bhaya D, Davison M, Barrangou R. CRISPR-Cas systems in bacteria and archaea: versatile small RNAs for adaptive defense and regulation. *Annu Rev Genet*, 2011, 45: 273-297.

16. Wiedenheft B, Sternberg S H, Doudna J A. RNA-guided genetic silencing systems in bacteria and archaea. *Nature*, 2012, 482(7385): 331-338.

17. Jinek M, Chylinski K, Fonfara I, et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, 2012, 337(6096): 816-821.

18. Cong L, Ran F A, Cox D, et al. Multiplex genome engineering using CRISPR/Cas systems. *Science*, 2013, 339(6121): 819-823.

19. Mali P, Yang L, Esvelt K M, et al. RNA-guided human genome engineering via Cas9. *Science*, 2013, 339(6121): 823-826.

20. Cho S W, Kim S, Kim J M, et al. Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. *Nat Biotechnol*, 2013, 31(3): 230-232.

21. Jinek M, East A, Cheng A, et al. RNA-programmed genome editing in human cells. *ELife*, 2013, 2: e00471.

22. Ran F A, Hsu P D, Lin C Y, et al. Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. *Cell*, 2013, 154(6): 1380-1389.

23. Guilinger J P, Thompson D B, Liu D R. Fusion of catalytically inactive Cas9 to FokI nuclease improves the specificity of genome modification. *Nat Biotechnol*, 2014, 32(6): 577-582.

24. Komor A C, Kim Y B, Packer M S, et al. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature*, 2016, 533(7603): 420-424.

25. Nishida K, Arazoe T, Yachie N, et al. Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems. *Science*, 2016, 353(6305), doi: 10.1126/science.aaf8729.

26. Ma Y, Zhang J, Yin W, et al. Targeted AID-mediated mutagenesis (TAM) enables efficient genomic diversification in mammalian cells. *Nat Methods*, 2016, 13(12): 1029-1035.

27. Gaudelli N M, Komor A C, Rees H A, et al. Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature*, 2017, 551(7681): 464-471.

28. Qi L S, Larson M H, Gilbert L A, et al. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell*, 2013, 152(5): 1173-1183.

29. Gilbert L A, Larson M H, Morsut L, et al. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell*, 2013, 154(2): 442-451.

30. Zetsche B, Gootenberg J S, Abudayyeh O O, et al. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell*, 2015, 163(3): 759-771.

31. Li X, Wang Y, Liu Y, et al. Base editing with a Cpf1-cytidine deaminase fusion. *Nat Biotechnol*, 2018, 36(4): 324-327.

32. Abudayyeh O O, Gootenberg J S, Konermann S, et al. C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. *Science*, 2016, 353(6299), doi: 10.1126/science.aaf5573.

33. Cox D B T, Gootenberg J S, Abudayyeh O O, et al. RNA editing with CRISPR-Cas13. *Science*, 2017, 358(6366): 1019-1027.

34. Gootenberg J S, Abudayyeh O O, Lee J W, et al. Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science*, 2017, 356(6336): 438-442.

35. Smargon A A, Cox D B T, Pyzocha N K, et al. Cas13b is a Type VI-B CRISPR-associated RNA-guided RNase differentially regulated by accessory proteins Csx27 and Csx28. *Mol Cell*, 2017, 65(4): 618-630.

36. Yan W X, Chong S R, Zhang H B, et al. Cas13d is a compact RNA-targeting Type VI CRISPR effector positively modulated by a WYL-domain-containing accessory protein. *Mol Cell*, 2018, 70(2): 327-339.

37. Wang H, La Russa M, Qi L S. CRISPR/Cas9 in genome editing and beyond. *Annu Rev Biochem*, 2016, 85: 227-264.

38. Dymond J S, Richardson S M, Coombes C E, et al. Synthetic chromosome arms function in yeast and generate phenotypic diversity by design. *Nature*, 2011, 477(7365): 471-476.

39. Jones S. SCRaMbLE does the yeast genome shuffle. *Nat Biotechnol*, 2018, 36(6): 503-503.

40. Shen M J, Wu Y, Yang K, et al. Heterozygous diploid and interspecies SCRaMbLEing. *Nat Commun*, 2018, 9(1): 1934.

41. Wu Y, Zhu R Y, Mitchell L A, et al. In vitro DNA SCRaMbLE. *Nat Commun*, 2018, 9(1): 1935.

42. Luo Z, Wang L, Wang Y, et al. Identifying and characterizing SCRaMbLEd synthetic yeast using ReSCuES. *Nat Commun*, 2018, 9(1): 1930.

43. Hochrein L, Mitchell L A, Schulz K, et al. L-SCRaMbLE as a tool for light-controlled Cre-mediated recombination in yeast. *Nat Commun*, 2018, 9(1): 1931.

44. Jia B, Wu Y, Li B Z, et al. Precise control of SCRaMbLE in synthetic haploid and diploid yeast. *Nat Commun*, 2018, 9(1): 1933.

45. Zhang W, Zhao Z, Luo Z, et al. Engineering the ribosomal DNA in a megabase synthetic chromosome. *Science*, 2017, 355(6329): eaaf3981.

46. Richardson S M, Mitchell L A, Stracquadanio G, et al. Design of a synthetic yeast genome. *Science*, 2017, 355(6329): 1040-1044.

47. Boeke J D, Church G, Hessel A, et al. The Genome Project-Write. *Science*, 2016, 353(6295): 126-127.

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