Gene Therapy (2020) 27:312–320
https://doi.org/10.1038/s41434-020-0181-5

COMMENT

Recent advances of genome editing and related technologies in China

Wen Sun[1,2] · Haoyi Wang[1,2,3]

Received: 21 February 2020 / Revised: 24 April 2020 / Accepted: 22 July 2020 / Published online: 3 August 2020

Abstract

Genome editing is a powerful tool, enabling scientists to alter DNA sequence at virtually any genome locus in any species. Different technologies have been developed employing programmable nucleases including meganuclease, zinc-finger nucleases, transcription activator-like effector nucleases, and most recently CRISPR-Cas systems. Chinese research groups are making important contributions at an increasing speed in genome editing field in recent years. In this review, we summarize recent progress made by Chinese scientists on the technological development of genome editing and beyond, focusing on the optimization and expanded application of existing genome editing tools, as well as the exploration of novel proteins as potential genome editing tools.

Introduction

Technology that enables precise and efficient manipulation of DNA sequences at desired genomic locus in living cells has long been pursued by biologists. Since 1970s, recombinant DNA technology has been used to engineer DNA fragments in test tubes with great precision [1–3]. However, precisely editing the DNA sequences within the genome of living cells has remained a formidable challenge until the establishment of genome editing technology. Genome editing is achieved with the advent of programmable nucleases that generate DNA double-stranded breaks (DSBs) at predefined genomic loci. First the meganucleases [4–6], zinc-finger nucleases (ZFNs) [7–9], then transcription activator-like effector nucleases (TALENs) [10–12], and most recently CRISPR-Cas systems (derived from clustered regularly interspaced short palindromic repeat (CRISPR) and CRISPR-associated (Cas) loci) serve as powerful tools to make targeted genomic alterations [13–15].

Remarkably, the CRISPR-Cas system, adaptive immune systems protecting prokaryotic organisms (pAgos) against invading phage infection, quickly became the most powerful and versatile platform for genome editing because of its diversity, robustness, and flexibility. In addition to the DNA cleavage capability of Cas effectors, catalytical inactive Cas enzymes (dCas) could be repurposed to achieve targeted gene regulation, epigenetic editing, and chromatin labeling [16–19]. Of note, repurposing the catalytical impaired nickase Cas9 enzymes (nCas9) or dead Cas9 (dCas9) generated the base editing (BE) platform, which catalyzes single nucleotide changes without introducing DNA DSBs.

As genome editing becoming one of the most attractive and competitive fields in science, Chinese scientific community has played a significant role in the application and development of the genome editing technology. A recent analysis of more than 2000 patent applications for distinct inventions that involved CRISPR indicated that the United States of America and China are neck and neck (USA's 872 versus China's 858, priority date up to 31 December 2017) [20]. As for the CRISPR-related publications, China is also close second to the United States of America, which has contributed the most publications (as of 27 March 2019) [21]. Of note, in some fields, such as agriculture and industrial applications, China takes the lead regarding the number of both the patents and papers. This review focuses

✉ Haoyi Wang
wanghaoyi@ioz.ac.cn

[1] State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, 100101 Beijing, China

[2] Institute for Stem Cell and Regeneration, Chinese Academy of Sciences, 100101 Beijing, China

[3] University of the Chinese Academy of Sciences, 100049 Beijing, China

on the technological development of genome editing and related technologies in China. Works on application of the technology and study of the molecular mechanisms are out of the scope of this review. Although we have striven to cover many primary studies, we apologize in advance to those whose work might have been unintentionally omitted.

Optimization of existing genome editing tools

TALENs, although easier to design than ZFNs, are still difficult to assemble molecularly, hindering their wider application. Several Chinese groups have developed methods to assemble customized TALE arrays efficiently [22–25]. For example, Yang et al.'s group developed uracil-specific excision reagent (USER)-based ligation mediated assembly of TAL effector system [24], which takes advantage of USER to make multiple different sticky ends between any neighboring DNA fragments for specific ligation. Multiple TALEs could be assembled within hours with preassembled templates [24]. The same group also conducted a thorough investigation of all possible repeat-variable diresidues (two hypervariable residues in each TALE module responsible for DNA binding) for their capability of recognizing DNA or chemically modified DNA (5-methylcatosine and 5-hydroxymethylcytosine) [26], greatly expanding the TALE platform.

The commonly used CRISPR-Cas9 system is composed of the Cas9 nuclease and a single-guide RNA (sgRNA) that carries a 20 nucleic acids sequence (also called protospacer) that is complementary to the target DNA. A short sequence called protospacer-adjacent motif (PAM) on the target sequence is also essential for Cas9 cleavage. Designing highly efficient sgRNAs with minimal off-target cleavage is crucial for effective application of CRISPR-Cas9 system. Chinese scientists developed multiple sgRNA design tools [27–35] and sgRNA selection systems [36, 37] for enhanced specificity and efficiency. Database CRISPRlnc was also established based on many manually validated sgRNAs, facilitating choosing sgRNAs with known efficiencies [38]. Strategies using multiple sgRNAs were developed to enhance gene editing efficiency in various applications and organisms [39–43]. In addition, the association of other sgRNA parameters such as length, structure, chemical modification, and expression level with the editing efficiency has also been studied [44–51].

Apart from sgRNA, researchers also put efforts on engineering Cas effectors to improve the fidelity and expand the targetable genomic sequences. Choi et al.'s group established a platform named CombiSEAL to systematically evaluate a library of 948 combination mutants of Streptococcus pyogenes Cas9 (SpCas9) nuclease for their genome editing activity. This work led to the identification of Opi-SpCas9, which possesses enhanced editing specificity and targeting range without compromising efficiency [52]. At the same time, another group using targeted mutagenesis in PAM interaction region of Staphylococcus aureus Cas9 (SaCas9) yielded several variants with altered PAM sequences, expanding targeting scope of SaCas9 [53].

Compared with Cas9, Cas12a (also called Cpf1) effectors have several unique characteristics: they do not require tracrRNA; PAM sequence is T-rich; generate DSB with over-hangs; have smaller sizes; and easier multiplexing due to inherent RNase activity for processing the crRNA array. Zetsche et al.'s group first revealed two Cas12a enzymes active in mammalian cells AsCpf1 and LbCpf1 [54]. However, the targeting scope of Cpf1 is limited by the long PAM (5′-TTTN-3′ PAM) requirement. Tu et al.'s group showed that FnCpf1 was active in mammalian cells with a simple 5′-TTN-3′ PAM, which only displayed in vitro nuclease activity previously [55]. More Cpf1 orthologs were identified by Teng et al.'s group, including one recognizing more flexible 5′YTN and 5′-TYYN PAMs, further expanding the scope of editable genome [56]. Though many novel Cpf1 orthologs were found, Cpf1 proteins has generally lower efficiency compared with Cas9. To increase efficiency of Cas12a for generating gene-modified rabbits and pigs, Wu et al. inserted a transfer RNA precursor sequence downstream of the crRNA protects it from digestion [57]. A modified crRNA scaffold through engineering the nucleotide substitutions at the loop region was also reported to increase Cas12a efficiency [56].

Systematic interpretation of gene function needs genome editing function in a robust and inducible manner. One way is to utilize estrogen receptor (ER) to achieve drug inducible control. Upon ligand binding, ER translocates from the cytoplasm to the nucleus where genomic DNA resides in. Based on hybrid inducible technologies (HIT), Zhao et al. fused ER to Cas9 and TALE leading to the development of HIT-Cas9 [58] and HIT-TALEN [59]. Apart from Cas9, sgRNA could also be controlled in an inducible manner to achieve temporal control of CRISPR-Cas9 system [60].

Expanded applications of existing genome editing technologies

One major advantage of the CRISPR-Cas system is the great flexibility of repurposing its function. The Cas9 protein harbors two nucleolytic domains (RuvC and HNH), each cleaving one specific target DNA strand, and together generating DSB [13]. Mutating single nucleolytic domain of Cas9 generates nCas9 that makes single-stranded DNA cuts, while Cas9 with both nucleolytic domains mutated becomes a programmable DNA-binding protein without

endonuclease activity called dCas9. dCas9 and nCas9 have significantly expanded the application of CRISPR-Cas9 technology. Of note, BE technology leveraging dCas9 or nCas9 fused with programmable deaminases is one of the most remarkable applications. In addition, dCas9 or TALENs fused with other different functional domains can bind to regulatory elements and regulate transcription, epigenetic modifications, as well as enable chromatin imaging in live cells [18]. Other expanded applications of genome editing technologies include high-throughput genetic screening, large-scale genome editing projects, and in vitro nucleic acid detection and assembly.

Base editing

BE is a novel approach that generates precise nucleotide changes in target DNA or RNA, without introducing DNA DSBs [61]. BE does not rely on homologous recombination, making single-nucleotide editing more efficient and precise, especially for postmitotic cells. Cytosine base editors (CBEs), first developed by Nishida et al.'s and Komor et al.'s groups, mediate C•G to T•A conversions. They are composed of a dCas9 or nCas9 fused to a single-stranded DNA deaminase [61, 62]. Later, Gaudelli et al.'s group developed adenine base editors (ABEs) capable of converting A•T to G•C, by fusing dCas9 or nCas9 to an adenine deaminase TadA, which was artificially evolved from a bacterial tRNA deaminase [63]. Although efficient and precise, the accessible sequences of CBEs and ABEs are highly dependent on the PAM sequences of Cas effectors. To circumvent this limitation, Chinese scientists replaced the nCas9 with catalytically inactive Cpf1 (dCpf1) [64] or various catalytically compromised Cas9 variants to increase the targeting scope of either CBE [65–70] or ABE [67–71]. Alternatively, the targeting scope of CBE could be expanded by diversified lamprey cytidine deaminases [72]. Other strategies to expand editing window were also reported, such as BE-PLUS, in which fusing ten copies of GCN4 peptide to nCas9 recruits scFv-APOBEC-UGI-GB1 to the target sites [73]. Considering the chromatin microenvironment as a possible obstacle for efficient editing, improving access to DNA through employing dCas9 binding to sequence about 20–30 base pair away from the target site also increased BE efficiency [74]. Through a human APOBEC3A–Cas9 fusion, efficient BE in methylated regions was also achieved [75].

In addition to DNA editing, site-specific RNA editing offers a new way to manipulate genetic information in a reversible and tunable manner. The key enzymes for RNA editing are adenosine deaminase acting on RNA (ADAR) adenosine deaminase, which makes adenosine-to-inosine conversion. Cox et al. first developed Cas-derived RNA base editors by fusing a catalytically inactive Cas13 (dCas13) and ADAR to make A-I conversions in mammalian cells [76]. While functional, this system poses challenges for the viral packaging and raises potential concerns of immunogenicity and oncogenicity [77, 78]. Qu et al.'s group established an approach called leveraging endogenous ADAR for programmable editing of RNA (LEAPER), which employs short engineered ADAR-recruiting RNAs to recruit endogenous ADAR enzymes to achieve RNA editing [79]. LEAPER enables simple, precise, and efficient RNA editing in a variety of cell types, without using any CRISPR-Cas system.

Though DNA and RNA BE methods enable precise and direct genome editing at single-base level, the issue of off-target edits remains a major concern. Chinese scientists developed several tools to evaluate the off-target effects of DNA base editors and provided modified versions of base editor with reduced off-target effects. Liang et al. developed EndoV-seq method to characterize the genome-wide off-target deamination by ABEs in human cells [80]. The relative high specificity of ABE was also validated by whole genome sequencing on mutant mice through treatment of ABE7.10 and sgRNA targeting *Hoxd13* locus in one-cell-stage embryo [81]. In contrast to ABE, numerous single-nucleotide variants were detected by CBE using a method called genome-wide off-target analysis by two-cell embryo injection by editing one blastomere of two-cell mouse embryos [82]. Similar results were obtained in rice [83]. Though ABEs showed high specificity at the DNA level, Zhou et al. found that both the CBE BE3 and the adenine base editor ABE7.10 generated tens of thousands of off-target RNA SNVs. In addition, they generated CBE and ABE variants with reduced RNA off-target mutation rate through protein engineering [84].

Transcription regulation and epigenome editing

Chinese scientists developed a variety of tools for transcription regulation and epigenome editing. Our group developed Casilio system by combining dCas9 and Pumilio RNA-binding protein [85], providing a versatile tool for studying gene function and chromosome structure through its capability of multiplexing and multimerization of effector proteins for gene regulation, epigenetic editing, and chromosomal labeling. Xu et al.'s group developed a strategy of recruiting trans enhancer to further enhance the level of transcription activation [86]. Zhan et al. established a method named "CRISPReader" that efficiently activates promoterless genes and constructs an all-in-one AAV-CRISPR-Cas9 system [87]. For easy packaging and delivery, catalytically inactive Cpf1 (dCpf1) [88], downsized

Cas9 [89], or logic circuits constructed by integrating multiple split dCas9 domains [90] were developed for transcriptional control. To achieve transcriptional control of multiple genes in vivo, Zhou et al. generated a transgenic mouse using an improved dCas9 system enabling simultaneous and precise in vivo transcriptional activation of multiple genes and long noncoding RNAs in the nervous system [91].

Large-scale epigenomic project such as the Encyclopedia of DNA elements [92, 93] and Roadmap Epigenome Mapping Consortium [94] provided valuable information on epigenetic modification across the genome in various cell lines and tissues. However, the functional study of the epigenome relies on the site-specific epigenome editing tools. Chinese scientists developed dCas9 based approach for targeted DNA demethylation [95] and RNA N-6-methyladenosine editing [96]. The genome-wide on-target and off-target properties were also determined for RNA-guided DNA methylation by dCas9 fused with methyltransferases [97]. In addition to dCas9, TALE protein for 5-methylcytosine and 5-hydroxymethylcytosine recognition was also developed, providing tools for TALE-dependent epigenome recognition [98].

Live cell chromatin imaging

The organization of chromatin structure in the 3D nuclear space is essential for mediating lineage-specific gene expression [99]. The conventional fluorescent in-situ hybridization methods have been playing a significant role for determining the nuclear positions of specific genomic loci; however, the requirement of cell fixation treatment prohibits its application for live cell genomic imaging [100].

The development of gene editing tools provides new strategies for live cell chromatin imaging. Ren et al.'s group developed fluorescently labeled TALEs fused with thioredoxin (TTALEs) to target and quantify the repetitive regions of the genome [101]. While dCas9 platform has been successfully applied to label repetitive telomeres and centromeres regions by dual-color chromatin imaging [102], live imaging of nonrepetitive regions is relatively challenging. Chen et al.'s group developed a CRISPR-Tag system to label endogenous protein-coding genes in living cells. This system enables simultaneous live imaging of both protein and DNA of human protein-coding genes. However, it requires knock in a tag within the gene of interest in the studied cells [103]. Ma et al. reported CRISPR-Sirius method, leveraging modified sgRNA scaffold to carry multiple fluorescent proteins binding sites [104]. Apart from relying on fluorescent proteins for genomic imaging, a molecular beacon (MB), and an sgRNA harboring a unique MB target sequence (sgRNA-MTS) was combined to generate CRISPR/MB. Dual-color imaging can also be achieved through two orthogonal MB/MTS pairs [105]. The sensitivity of CRISPR/MB is further improved through modifying sgRNA to carry two distinct MBs that can undergo fluorescence resonance energy transfer [106].

High-throughput genetic screening

In addition to the technology editing individual genes, large-scale loss of function genetic screen is a powerful tool for studying biology and disease. Zhou et al. and Zhu et al. developed high-throughput screening methods for both protein-coding genes and long noncoding RNAs in human cells using a sgRNA library or a lentiviral paired-guide RNA library, respectively [107, 108]. Recently, they reported a sgRNA design strategy with embedded barcodes facilitating CRISPR-pooled screening [109]. Zhong et al.'s group conducted a CRISPR-Cas-based genetic screening in vivo using haploid embryonic cells carrying a sgRNA library [110]. CRISPR screening has been combined with single-cell RNAseq to enable functional screening at a single-cell level. Duan et al. developed an analytical tool MUSIC for single-cell CRISPR screening data analysis [111].

Instead of gene disruption-based screening methods, generation of gain-of-function mutations by making nucleotide changes provides strategies for directed evolution. In addition to the APOBEC adenosine deaminase enzymes mentioned above, the activation-induced adenosine deaminase (AID) could also been fused to the dCas protein. Guided by sgRNA, this dCas-AID complex directly changed C or G to the other three bases, generating a variants repertoire at target site. Groups of Ma et al. and Ren et al. established this technique in mammalian [112] and rice cells [113] to achieve targeted random mutagenesis. Very recently, a more complex dual cytosine and ABEs were developed by Li et al. for the same purpose [114]. In contrast with CRISPR-Cas based functional screening through gene disruption or expression regulation, these targeted AID-mediated mutagenesis offers a forward genetic tool to screen for gain-of-function variants at single-base resolution.

Genome editing related large-scale project

With the fast advancement of genome editing technology, Chinese scientists start to launch large-scale genome editing projects. Led by Anming Meng, a project involving 38 laboratories from 24 institutions in China was initiated in 2013, aiming to knock out each annotated gene on zebrafish Chromosome 1. This work was recently finished, providing an invaluable resource to the zebrafish research community [115]. Another large-scale project is the genome tagging

project, led by Jinsong Li, planning to tag every protein-coding gene in mice, which will greatly facilitate the illustration of proteins' localization and physical interaction in vivo. This project utilizes the CRISPR-Cas9-based genome editing technology and the androgenic haploid ESCs to generate gene-modified semi-cloned mice [116].

Tools for in vitro nucleic acid detection and assembly

Besides applications for genome editing and transcription regulation, CRISPR-Cas system could also be harnessed for diagnostics such as detection of the nucleic acid from bacterial and viral pathogens. Li et al. identified the ssDNA collateral activity of Cas12a triggered by specific binding of DNA [117, 118]. Based on this feature, this group developed an one-hour low-cost multipurpose highly efficient system for fast detection of target DNA as well as target RNA, using Cas12a [119]. Using other Cas12 orthologs for nucleic acid detection were subsequently reported by several Chinese research groups [120–124]. Apart from Cas12, Cas9 [125–127], and TALEN [128] have also been harnessed for nucleic acid detection. In addition to nucleic acid detection, Chinese scientists also applied Cas12a or Cas9 for DNA constructs assembly [129–131] or site-directed mutagenesis [132].

Exploration of novel proteins (orthologs) as potential genome editing tools

Searching for novel gene editing systems gained increasing interests due to the great diversity of natural enzymes related to nucleic acid. Different from Cas12a, Cas12b is a dual-RNA-guided nuclease. Although the initially identified Cas12b (AaCas12b) displayed optimal cleavage activity in vitro at the temperature of 48 °C [133], precluding its application to mammalian genome editing, deeper bioinformatic exploration by Teng et al.'s group led to the discovery of *Alicyclobacillus acidiphilus* (AaCas12b), establishing a third class of RNA-guided gene editing platform after Cas9 and Cas12a [134]. AaCas12b demonstrated versatile applications including single and multiplex genome editing, as well as gene activation. Moreover, AaCas12b showed greater specificity compared with SpCas9, holding potential advantage for therapeutic applications.

Apart from the major gene editing platforms ZNFs, TALENs, and CRISPR-Cas, Argonaute proteins from pAgos are involved in nucleic acid-guided host defense against invading nucleic acids, holding potential for being developed into novel gene editing technology [135]. Our group recently reported two pAgos that could catalyze DNA-guided cleavage of single- and double-stranded DNA at 37 °C [136]. Although only in vitro activity was displayed, these findings raise exciting possibility of exploiting pAgos as novel tools. Another novel DNA editing tool called structure-guided endonuclease was reported, while further studies are desirable to characterize the system, such as demonstrating wider applications and defining potential off-target effects [137].

Conclusion

Genome editing technology, particularly the CRISPR-Cas system, has revolutionized biology research. During the recent years, we have witnessed the amazing progress in the development of various tools for genome editing and beyond. China is making increasing contribution to this field. This review mainly focuses on the contributions of technology development made by Chinese research groups. For more comprehensive review of the entire field please refer to several excellent reviews published recently [138–140].

Without doubt, further exploration of the natural diversity will lead to the development of novel biotechnologies with distinct properties. We believe that, with increasing investment and growing interests, more exciting technologies for gene editing and beyond will emerge from China, making even more contributions to the international science community.

Acknowledgements HW is supported by the National Key Research and Development Program of China (Nos 2018YFA0107703, 2019YFA0110000), Strategic Priority Research Program of the Chinese Academy of Sciences (No. XDA16010503), and National Natural Science Foundation of China (No. 31722036). WS is supported by the National Key Research and Development Program of China (No. 2018YFE0201102).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Danna K, Nathans D. Specific cleavage of simian virus 40 DNA by restriction endonuclease of *Haemophilus influenzae*. Proc Natl Acad Sci USA. 1971;68:2913–7.
2. Kelly TJ, Smith HO. A restriction enzyme from *Haemophilus influenzae*: II. Base sequence of the recognition site. J Mol Biol. 1970;51:393–409.
3. Smith HO, Wilcox KW. A restriction enzyme from *Haemophilus influenzae*. I. Purification and general properties. J Mol Biol. 1970;51:379–91.
4. Stoddard BL. Homing endonuclease structure and function. Q Rev Biophys. 2005;38:49–95.

5. Silva G, Poirot L, Galetto R, Smith J, Montoya G, Duchateau P, et al. Meganucleases and other tools for targeted genome engineering: perspectives and challenges for gene therapy. Curr Gene Ther. 2011;11:11–27.
6. Stoddard BL. Homing endonucleases from mobile group I introns: discovery to genome engineering. Mobile DNA. 2014;5:7.
7. Kim YG, Cha J, Chandrasegaran S. Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain. Proc Natl Acad Sci USA. 1996;93:1156–60.
8. Cathomen T, Joung JK. Zinc-finger nucleases: the next generation emerges. Mol Ther. 2008;16:1200–7.
9. Carroll D. Genome engineering with zinc-finger nucleases. Genetics. 2011;188:773–82.
10. Christian M, Cermak T, Doyle EL, Schmidt C, Zhang F, Hummel A, et al. Targeting DNA double-strand breaks with TAL effector nucleases. Genetics. 2010;186:757–61.
11. Boch J, Scholze H, Schornack S, Landgraf A, Hahn S, Kay S, et al. Breaking the code of DNA binding specificity of TAL-type III effectors. Science. 2009;326:1509–12.
12. Moscou MJ, Bogdanove AJ. A simple cipher governs DNA recognition by TAL effectors. Science. 2009;326:1501.
13. 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;337:816–21.
14. Koonin EV, Makarova KS. Origins and evolution of CRISPR-Cas systems. Philos Trans R Soc Lond B Biol Sci. 2019;374:20180087.
15. Ishino Y, Krupovic M, Forterre P. History of CRISPR-Cas from encounter with a mysterious repeated sequence to genome editing technology. J Bacteriol. 2018;200:e00580–17.
16. Lo A, Qi L. Genetic and epigenetic control of gene expression by CRISPR-Cas systems. F1000Res. 2017;6:1–16.
17. Pulecio J, Verma N, Mejia-Ramirez E, Huangfu D, Raya A. CRISPR/Cas9-based engineering of the epigenome. Cell Stem Cell. 2017;21:431–47.
18. Xu X, Qi LS. A CRISPR-dCas toolbox for genetic engineering and synthetic biology. J Mol Biol. 2019;431:34–47.
19. Brezgin S, Kostyusheva A, Kostyushev D, Chulanov V. Dead Cas systems: types, principles, and applications. Int J Mol Sci. 2019;20:6041.
20. Martin-Laffon J, Kuntz M, Ricroch AE. Worldwide CRISPR patent landscape shows strong geographical biases. Nat Biotechnol. 2019;37:613–20.
21. Huang Y, Porter A, Zhang Y, Barrangou R. Collaborative networks in gene editing. Nat Biotechnol. 2019;37:1107–9.
22. Zhang SY, Chen HT, Wang JK. Generate TALE/TALEN as easily and rapidly as generating CRISPR. Mol Ther. 2019;13:310–20.
23. Zhao JL, Sun WY, Liang J, Jiang J, Wu Z. A one-step system for convenient and flexible assembly of transcription activator-like effector nucleases (TALENs). Mol Cells. 2016;39:687–91.
24. Yang J, Yuan P, Wen D, Sheng Y, Zhu S, Yu Y, et al. ULtiMATE system for rapid assembly of customized TAL effectors. PloS ONE. 2013;8:e75649.
25. Zhang ZQ, Li D, Xu HR, Xin Y, Zhang TT, Ma LX, et al. A simple and efficient method for assembling TALE protein based on plasmid library. PloS ONE. 2013;8:e66459.
26. Yang J, Zhang Y, Yuan P, Zhou Y, Cai C, Ren Q, et al. Complete decoding of TAL effectors for DNA recognition. Cell Res. 2014;24:628–31.
27. Zhao CZ, Zheng XG, Qu WB, Li GL, Li XY, Miao YL, et al. CRISPR-offinder: a CRISPR guide RNA design and off-target searching tool for user-defined protospacer adjacent motif. Int J Biol Sci. 2017;13:1470–8.
28. Yan JF, Chuai GH, Zhou C, Zhu CY, Yang J, Zhang C, et al. Benchmarking CRISPR on-target sgRNA design. Brief Bioinform. 2018;19:721–4.
29. Sun JM, Liu H, Liu JX, Cheng SK, Peng Y, Zhang QH, et al. CRISPR-local: a local single-guide RNA (sgRNA) design tool for non-reference plant genomes. Bioinformatics. 2019; 35:2501–3.
30. Chuai GH, Ma HH, Yan JF, Chen M, Hong NF, Xue DY, et al. DeepCRISPR: optimized CRISPR guide RNA design by deep learning. Genome Biol. 2018;19:80.
31. Guo JH, Wang TM, Guan CG, Liu B, Luo C, Xie Z, et al. Improved sgRNA design in bacteria via genome-wide activity profiling. Nucleic Acids Res. 2018;46:7052–69.
32. Xue L, Tang B, Chen W, Luo JS. Prediction of CRISPR sgRNA activity using a deep convolutional neural network. J Chem Inf Model. 2019;59:615–24.
33. Xie SS, Shen B, Zhang CB, Huang XX, Zhang YL. sgRNAcas9: a software package for designing CRISPR sgRNA and evaluating potential off-target cleavage sites. PloS ONE. 2014;9: e100448.
34. Wang L, Zhang JH. Prediction of sgRNA on-target activity in bacteria by deep learning. Bmc Bioinform. 2019;20:517.
35. Xiong YY, Xie XW, Wang YZ, Ma WB, Liang PP, Zhou SY, et al. pgRNAFinder: a web-based tool to design distance independent paired-gRNA. Bioinformatics. 2017;33:3642–4.
36. Zhang HW, Zhang XX, Fan CX, Xie Q, Xu CX, Zhao Q, et al. A novel sgRNA selection system for CRISPR-Cas9 in mammalian cells. Biochem Biophys Res Commun. 2016;471:528–32.
37. Liang G, Zhang HM, Lou DJ, Yu DQ. Selection of highly efficient sgRNAs for CRISPR/Cas9-based plant genome editing. Sci Rep. 2016;6:21451.
38. Chen W, Zhang GQ, Li J, Zhang X, Huang SL, Xiang SL, et al. CRISPRlnc: a manually curated database of validated sgRNAs for lncRNAs. Nucleic Acids Res. 2019;47:D63–8.
39. Zhou JK, Wang JY, Shen B, Chen L, Su Y, Yang J, et al. Dual sgRNAs facilitate CRISPR/Cas9-mediated mouse genome targeting. FEBS J. 2014;281:1717–25.
40. Wu JJ, Tang Y, Zhang CL. Targeting N-terminal huntingtin with a dual-sgRNA strategy by CRISPR/Cas9. Biomed Res Int. 2019;2019:1039623.
41. Tang YD, Guo JC, Wang TY, Zhao K, Liu JT, Gao JC, et al. CRISPR/Cas9-mediated 2-sgRNA cleavage facilitates pseudorabies virus editing. FASEB J. 2018;32:4293–301.
42. Chen XY, Xu F, Zhu CM, Ji JJ, Zhou XF, Feng XZ, et al. Dual sgRNA-directed gene knockout using CRISPR/Cas9 technology in Caenorhabditis elegans. Sci Rep. 2014;4:7581.
43. Zuo E, Cai YJ, Li K, Wei Y, Wang BA, Sun Y, et al. One-step generation of complete gene knockout mice and monkeys by CRISPR/Cas9-mediated gene editing with multiple sgRNAs. Cell Res. 2017;27:933–45.
44. Zhang JP, Li XL, Neises A, Chen WQ, Hu LP, Ji GZ, et al. Different effects of sgRNA length on CRISPR-mediated gene knockout efficiency. Sci Rep. 2016;6:28566.
45. Xu JY, Lian W, Jia YN, Li LY, Huang Z. Optimized guide RNA structure for genome editing via Cas9. Oncotarget. 2017;8:94166–71.
46. Long L, Guo DD, Gao W, Yang WW, Hou LP, Ma XN, et al. Optimization of CRISPR/Cas9 genome editing in cotton by improved sgRNA expression. Plant Methods. 2018;14:85.
47. Liu WQ, Li SF, Zhang YB, Li JS, Li YP. Efficient CRISPR-based genome editing using tandem guide RNAs and editable surrogate reporters. FEBS Open Bio. 2018;8:1167–75.
48. Zhang DB, Zhang HW, Li TD, Chen KL, Qiu JL, Gao CX. Perfectly matched 20-nucleotide guide RNA sequences enable robust genome editing using high-fidelity SpCas9 nucleases. Genome Biol. 2017;18:191.

49. Mu W, Zhang Y, Xue X, Liu L, Wei X, Wang H. 5' capped and 3' polyA-tailed sgRNAs enhance the efficiency of CRISPR-Cas9 system. Protein Cell. 2019;10:223–8.
50. Mu W, Tang N, Cheng C, Sun W, Wei X, Wang H. In vitro transcribed sgRNA causes cell death by inducing interferon release. Protein Cell. 2019;10:461–5.
51. Ren XJ, Yang ZH, Xu J, Sun J, Mao DC, Hu YH, et al. Enhanced specificity and efficiency of the CRISPR/Cas9 system with optimized sgRNA parameters in Drosophila. Cell Rep. 2014;9:1151–62.
52. Choi GCG, Zhou P, Yuen CTL, Chan BKC, Xu F, Bao S, et al. Combinatorial mutagenesis en masse optimizes the genome editing activities of SpCas9. Nat Methods. 2019;16:722–30.
53. Ma DC, Xu ZM, Zhang ZY, Chen X, Zeng XZ, Zhang YY, et al. Engineer chimeric Cas9 to expand PAM recognition based on evolutionary information. Nat Commun. 2019;10:560.
54. Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, et al. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell. 2015;163:759–71.
55. Tu M, Lin L, Cheng Y, He X, Sun H, Xie H, et al. A 'new lease of life': FnCpf1 possesses DNA cleavage activity for genome editing in human cells. Nucleic Acids Res. 2017;45:11295–304.
56. Teng F, Li J, Cui TT, Xu K, Guo L, Gao QQ, et al. Enhanced mammalian genome editing by new Cas12a orthologs with optimized crRNA scaffolds. Genome Biol. 2019;20:15.
57. Wu H, Liu QS, Shi H, Xie JK, Zhang QJ, Ouyang Z, et al. Engineering CRISPR/Cpf1 with tRNA promotes genome editing capability in mammalian systems. Cell Mol Life Sci. 2018;75:3593–607.
58. Zhao C, Zhao YZ, Zhang JF, Lu J, Chen L, Zhang Y, et al. HIT-Cas9: a CRISPR/Cas9 genome-editing device under tight and effective drug control. Mol Ther Nucleic Acids. 2018;13:208–19.
59. Zhao C, Zhang Y, Zhao YZ, Ying Y, Ai RN, Zhang JF, et al. Multiple chemical inducible Tal effectors for genome editing and transcription activation. ACS Chem Biol. 2018;13:609–17.
60. Lin B, An Y, Meng L, Zhang H, Song J, Zhu Z, et al. Control of CRISPR-Cas9 with small molecule-activated allosteric aptamer regulating sgRNAs. Chem Commun. 2019;55:12223–6.
61. Nishida K, Arazoe T, Yachie N, Banno S, Kakimoto M, Tabata M, et al. Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems. Science. 2016;353: aaf8729.
62. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature. 2016;533:420–4.
63. Gaudelli NM, Komor AC, Rees HA, Packer MS, Badran AH, Bryson DI, et al. Programmable base editing of A*T to G*C in genomic DNA without DNA cleavage. Nature. 2017;551:464–71.
64. Li X, Wang Y, Liu Y, Yang B, Wang X, Wei J, et al. Base editing with a Cpf1-cytidine deaminase fusion. Nat Biotechnol. 2018;36:324–7.
65. Zhang YH, Qin W, Lu XC, Xu JS, Huang HG, Bai HP, et al. Programmable base editing of zebrafish genome using a modified CRISPR-Cas9 system. Nat Commun. 2017;8:118.
66. Wu Y, Xu W, Wang FP, Zhao S, Feng F, Song JL, et al. Increasing cytosine base editing scope and efficiency with engineered Cas9-PmCDA1 fusions and the modified sgRNA in rice. Front Genet. 2019;10:379.
67. Qin R, Li J, Li H, Zhang Y, Liu X, Miao Y, et al. Developing a highly efficient and wildly adaptive CRISPR-SaCas9 toolset for plant genome editing. Plant Biotechnol J. 2019;17:706–8.
68. Liu XY, Li GL, Zhou XL, Qiao YB, Wang RX, Tang SH, et al. Improving editing efficiency for the sequences with NGH PAM using xCas9-derived base editors. Mol Ther Nucleic Acids. 2019;17:626–35.
69. Hua K, Tao XP, Zhu JK. Expanding the base editing scope in rice by using Cas9 variants. Plant Biotechnol J. 2019; 17:499–504.
70. Zeng D, Li X, Huang J, Li Y, Cai S, Yu W, et al. Engineered Cas9 variant tools expand targeting scope of genome and base editing in rice. Plant Biotechnol J. 2019;18:1348–50.
71. Yang L, Zhang X, Wang L, Yin S, Zhu B, Xie L, et al. Increasing targeting scope of adenosine base editors in mouse and rat embryos through fusion of TadA deaminase with Cas9 variants. Protein Cell. 2018;9:814–9.
72. Cheng TL, Li S, Yuan B, Wang XL, Zhou WH, Qiu ZL. Expanding C-T base editing toolkit with diversified cytidine deaminases. Nat Commun. 2019;10:3612.
73. Jiang W, Feng S, Huang S, Yu W, Li G, Yang G, et al. BE-PLUS: a new base editing tool with broadened editing window and enhanced fidelity. Cell Res. 2018;28:855–61.
74. Liu Y, Li G, Yang G, Gu H, Huang S, Yu W, et al. Increasing the targeting scope and efficiency of base editing with Proxy-BE strategy. FEBS Lett. 2019;594:1319–28.
75. Wang X, Li J, Wang Y, Yang B, Wei J, Wu J, et al. Efficient base editing in methylated regions with a human APOBEC3A-Cas9 fusion. Nat Biotechnol. 2018;36:946–9.
76. Cox DBT, Gootenberg JS, Abudayyeh OO, Franklin B, Kellner MJ, Joung J, et al. RNA editing with CRISPR-Cas13. Science. 2017;358:1019–27.
77. Chew WL, Tabebordbar M, Cheng JK, Mali P, Wu EY, Ng AH, et al. A multifunctional AAV-CRISPR-Cas9 and its host response. Nat Methods. 2016;13:868–74.
78. Teoh PJ, An O, Chung TH, Chooi JY, Toh SHM, Fan S, et al. Aberrant hyperediting of the myeloma transcriptome by ADAR1 confers oncogenicity and is a marker of poor prognosis. Blood. 2018;132:1304–17.
79. Qu L, Yi Z, Zhu S, Wang C, Cao Z, Zhou Z, et al. Programmable RNA editing by recruiting endogenous ADAR using engineered RNAs. Nat Biotechnol. 2019;37:1380.
80. Liang P, Xie X, Zhi S, Sun H, Zhang X, Chen Y, et al. Genome-wide profiling of adenine base editor specificity by EndoV-seq. Nat Commun. 2019;10:67.
81. Liu Z, Lu Z, Yang G, Huang S, Li G, Feng S, et al. Efficient generation of mouse models of human diseases via ABE- and BE-mediated base editing. Nat Commun. 2018;9:2338.
82. Zuo E, Sun Y, Wei W, Yuan T, Ying W, Sun H, et al. Cytosine base editor generates substantial off-target single-nucleotide variants in mouse embryos. Science. 2019;364:289–92.
83. Jin S, Zong Y, Gao Q, Zhu Z, Wang Y, Qin P, et al. Cytosine, but not adenine, base editors induce genome-wide off-target mutations in rice. Science. 2019;364:292–5.
84. Zhou C, Sun Y, Yan R, Liu Y, Zuo E, Gu C, et al. Off-target RNA mutation induced by DNA base editing and its elimination by mutagenesis. Nature. 2019;571:275–8.
85. Cheng AW, Jillette N, Lee P, Plaskon D, Fujiwara Y, Wang W, et al. Casilio: a versatile CRISPR-Cas9-Pumilio hybrid for gene regulation and genomic labeling. Cell Res. 2016;26:254–7.
86. Xu XH, Gao JL, Dai W, Wang DY, Wu J, Wang JK. Gene activation by a CRISPR-assisted trans enhancer. Elife. 2019;8: e45973.
87. Zhan HJ, Zhou Q, Gao QJ, Li JF, Huang WR, Liu YC. Multiplexed promoterless gene expression with CRISPReader. Genome Biol. 2019;20:113.
88. Zhang XC, Wang JM, Cheng QX, Zheng X, Zhao GP, Wang J. Multiplex gene regulation by CRISPR-ddCpf1. Cell Discov. 2017;3:17018.

89. Ma DC, Peng SG, Huang WR, Cai ZM, Xie Z. Rational design of mini-Cas9 for transcriptional activation. ACS Synth Biol. 2018;7:978–85.
90. Ma D, Peng S, Xie Z. Integration and exchange of split dCas9 domains for transcriptional controls in mammalian cells. Nat Commun. 2016;7:13056.
91. Zhou H, Liu J, Zhou C, Gao N, Rao Z, Li H, et al. In vivo simultaneous transcriptional activation of multiple genes in the brain using CRISPR-dCas9-activator transgenic mice. Nat Neurosci. 2018;21:440–6.
92. Consortium EP. An integrated encyclopedia of DNA elements in the human genome. Nature. 2012;489:57–74.
93. Davis CA, Hitz BC, Sloan CA, Chan ET, Davidson JM, Gabdank I, et al. The encyclopedia of DNA elements (ENCODE): data portal update. Nucleic Acids Res. 2018;46:D794–801.
94. Bernstein BE, Stamatoyannopoulos JA, Costello JF, Ren B, Milosavljevic A, Meissner A, et al. The NIH roadmap epigenomics mapping consortium. Nat Biotechnol. 2010;28:1045–8.
95. Xu XX, Tao YH, Gao XB, Zhang L, Li XF, Zou WG, et al. A CRISPR-based approach for targeted DNA demethylation. Cell Discov. 2016;2:16009.
96. Liu X-M, Zhou J, Mao Y, Ji Q, Qian S-B. Programmable RNA N6-methyladenosine editing by CRISPR-Cas9 conjugates. Nat Chem Biol. 2019;15:865–71.
97. Lin L, Liu Y, Xu FP, Huang JR, Daugaard TF, Petersen TS, et al. Genome-wide determination of on-target and off-target characteristics for RNA-guided DNA methylation by dCas9 methyltransferases. Gigascience. 2018;7:1–19.
98. Zhang Y, Liu LL, Guo SJ, Song JH, Zhu CX, Yue ZW, et al. Deciphering TAL effectors for 5-methylcytosine and 5-hydroxymethylcytosine recognition. Nat Commun. 2017;8:901.
99. Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, et al. Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature. 2012;485:376–80.
100. Bickmore WA. The spatial organization of the human genome. Annu Rev Genom Hum Genet. 2013;14:67–84.
101. Ren R, Deng L, Xue Y, Suzuki K, Zhang W, Yu Y, et al. Visualization of aging-associated chromatin alterations with an engineered TALE system. Cell Res. 2017;27:483–504.
102. Shao SP, Zhang WW, Hu H, Xue BX, Qin JS, Sun CY, et al. Long-term dual-color tracking of genomic loci by modified sgRNAs of the CRISPR/Cas9 system. Nucleic Acids Res. 2016;44:e86.
103. Chen BH, Zou W, Xu HY, Liang Y, Huang B. Efficient labeling and imaging of protein-coding genes in living cells using CRISPR-Tag. Nat Commun. 2018;9:5065.
104. Ma H, Tu LC, Naseri A, Chung YC, Grunwald D, Zhang S, et al. CRISPR-Sirius: RNA scaffolds for signal amplification in genome imaging. Nat Methods. 2018;15:928–31.
105. Wu XT, Mao SQ, Yang YT, Rushdi MN, Krueger CJ, Chen AK. A CRISPR/molecular beacon hybrid system for live-cell genomic imaging. Nucleic Acids Res. 2018;46:e80.
106. Mao SQ, Ying YC, Wu XT, Krueger CJ, Chen AK. CRISPR/dual-FRET molecular beacon for sensitive live-cell imaging of non-repetitive genomic loci. Nucleic Acids Res. 2019;47:e131.
107. Zhou Y, Zhu S, Cai C, Yuan P, Li C, Huang Y, et al. High-throughput screening of a CRISPR/Cas9 library for functional genomics in human cells. Nature. 2014;509:487–91.
108. Zhu S, Li W, Liu J, Chen CH, Liao Q, Xu P, et al. Genome-scale deletion screening of human long non-coding RNAs using a paired-guide RNA CRISPR-Cas9 library. Nat Biotechnol. 2016;34:1279–86.
109. Zhu SY, Cao ZZ, Liu ZH, He Y, Wang YA, Yuan PF, et al. Guide RNAs with embedded barcodes boost CRISPR-pooled screens. Genome Biol. 2019;20:20.
110. Zhong CQ, Yin Q, Xie ZF, Bai MZ, Dong R, Tang W, et al. CRISPR-Cas9-mediated genetic screening in mice with haploid embryonic stem cells carrying a guide RNA library. Cell Stem Cell. 2015;17:221–32.
111. Duan B, Zhou C, Zhu CY, Yu YF, Li GY, Zhang SH, et al. Model-based understanding of single-cell CRISPR screening. Nat Commun. 2019;10:2233.
112. Ma Y, Zhang J, Yin W, Zhang Z, Song Y, Chang X. Targeted AID-mediated mutagenesis (TAM) enables efficient genomic diversification in mammalian cells. Nat Methods. 2016;13:1029–35.
113. Ren B, Yan F, Kuang YJ, Li N, Zhang DW, Zhou XP, et al. Improved base editor for efficiently inducing genetic variations in rice with CRISPR/Cas9-guided hyperactive hAID mutant. Mol Plant. 2018;11:623–6.
114. Li C, Zhang R, Meng X, Chen S, Zong Y, Lu C, et al. Targeted, random mutagenesis of plant genes with dual cytosine and adenine base editors. Nat Biotechnol. 2020;38:875–82.
115. Sun Y, Zhang B, Luo L, Shi DL, Wang H, Cui Z, et al. Systematic genome editing of the genes on zebrafish Chromosome 1 by CRISPR/Cas9. Genome Res. 2019;30:118–26.
116. Jiang J, Yan M, Li D, Li J. Genome tagging project: tag every protein in mice through 'artificial spermatids'. Natl Sci Rev. 2018;6:394–6.
117. Li SY, Cheng QX, Liu JK, Nie XQ, Zhao GP, Wang J. CRISPR-Cas12a has both cis- and trans-cleavage activities on single-stranded DNA. Cell Res. 2018;28:491–3.
118. Chen JS, Ma E, Harrington LB, Da Costa M, Tian X, Palefsky JM, et al. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. Science. 2018;360:436–9.
119. Li SY, Cheng QX, Wang JM, Li XY, Zhang ZL, Gao S, et al. CRISPR-Cas12a-assisted nucleic acid detection. Cell Discov. 2018;4:20.
120. Bai J, Lin HS, Li HJ, Zhou Y, Liu JS, Zhong GR, et al. Cas12a-based on-site and rapid nucleic acid detection of African swine fever. Front Microbiol. 2019;10:2830.
121. Li LX, Li SY, Wu N, Wu JC, Wang G, Zhao GP, et al. HOLMESv2: a CRISPR-Cas12b-assisted platform for nucleic acid detection and DNA methylation quantitation. ACS Synth Biol. 2019;8:2228–37.
122. Teng F, Guo L, Cui TT, Wang XG, Xu K, Gao QQ, et al. CDetection: CRISPR-Cas12b-based DNA detection with sub-attomolar sensitivity and single-base specificity. Genome Biol. 2019;20:132.
123. Wang B, Wang R, Wang DQ, Wu J, Li JX, Wang J, et al. Cas12aVDet: a CRISPR/Cas12a-based platform for rapid and visual nucleic acid detection. Anal Chem. 2019;91:12156–61.
124. Zhao X, Zhang W, Qiu X, Mei Q, Luo Y, Fu W. Rapid and sensitive exosome detection with CRISPR/Cas12a. Anal Bioanal Chem. 2020;412:601–9.
125. Hu JY, Jiang M, Liu R, Lv Y. Label-Free CRISPR/Cas9 assay for site-specific nucleic acid detection. Anal Chem. 2019;91:10870–8.
126. Qiu XY, Zhu LY, Zhu CS, Ma JX, Hou T, Wu XM, et al. Highly effective and low-cost microRNA detection with CRISPR-Cas9. ACS Synth Biol. 2018;7:807–13.
127. Wang Q, Zhang BB, Xu XH, Long FF, Wang JK. CRISPR-typing PCR (ctPCR), a new Cas9-based DNA detection method. Sci Rep. 2018;8:14126.
128. Wang Y, Bai JL, Qu XC, Gao YF, Wang J, Li S, et al. High-specificity double-stranded DNA detection with a "humanoid" molecular beacon and TALEs. Nanoscale. 2018;10:18354–61.
129. Lei C, Li SY, Liu JK, Zheng X, Zhao GP, Wang J. The CCTL (Cpf1-assisted cutting and Taq DNA ligase-assisted Ligation) method for efficient editing of large DNA constructs in vitro. Nucleic Acids Res. 2017;45:e74.

130. Li SY, Zhao GP, Wang J. C-Brick: a new standard for assembly of biological parts using Cpf1. ACS Synth Biol. 2016;5:1383–8.
131. Wang LP, Wang HJ, Liu HY, Zhao QY, Liu B, Wang L, et al. Improved CRISPR-Cas12a-assisted one-pot DNA editing method enables seamless DNA editing. Biotechnol Bioeng. 2019;116:1463–74.
132. She WW, Ni J, Shui K, Wang F, He RY, Xue JH, et al. Rapid and error-free site-directed mutagenesis by a PCR-free in vitro CRISPR/Cas9-mediated mutagenic system. ACS Synth Biol. 2018;7:2236–44.
133. Shmakov S, Abudayyeh OO, Makarova KS, Wolf YI, Gootenberg JS, Semenova E, et al. Discovery and functional characterization of diverse class 2 CRISPR-Cas systems. Mol Cell. 2015;60:385–97.
134. Teng F, Cui TT, Feng GH, Guo L, Xu K, Gao QQ, et al. Repurposing CRISPR-Cas12b for mammalian genome engineering. Cell Discov. 2018;4:63.
135. Hegge JW, Swarts DC, van der Oost J. Prokaryotic argonaute proteins: novel genome-editing tools? Nat Rev Microbiol. 2017;16:5–11.
136. Cao Y, Sun W, Wang J, Sheng G, Xiang G, Zhang T, et al. Argonaute proteins from human gastrointestinal bacteria catalyze DNA-guided cleavage of single- and double-stranded DNA at 37 degrees C. Cell Discov. 2019;5:38.
137. Xu S, Cao SS, Zou BJ, Yue YY, Gu C, Chen X, et al. An alternative novel tool for DNA editing without target sequence limitation: the structure-guided nuclease. Genome Biol. 2016;17:186.
138. Adli M. The CRISPR tool kit for genome editing and beyond. Nat Commun. 2018;9:1911.
139. Zhang F. Development of CRISPR-Cas systems for genome editing and beyond. Q Rev Biophys. 2019;52:1–31.
140. Rees HA, Liu DR. Base editing: precision chemistry on the genome and transcriptome of living cells. Nat Rev Genet. 2018;19:770–788.