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# CRISPR CAS-9AGENE EDITING

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## ABSTRACT

The CRISPR-Cas9 (clustered often interspaced short palindromic repeats with CRISPR-associated macromolecule 9) system could be a ordering piece of writing system that's straightforward to style, extremely specific, efficient, and robust, for multiplexed gene editing and high output for a variety of cell types and organisms. In this article, recent applications of recent system, that is developing wide and more and more is being implement to by selection management organic phenomenon on a genome- wide scale.

## KEYWORDS

Genome editing, CRISPR and Gene expression.

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## INTRODUCTION

### Abbreviations

ZFN: Zinc Finger Nucleases; TALEN: Transcription Activator-Like Effector Nucleases; CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats; Cas: CRISPR Associated Endonuclease; ZFP: Zinc Finger Peptide; DSB: Double Stranded Break; sgRNA: Single Guide RNA; crRNA: Crispr RNA; trRNA-Trans Activating RNA; PAM: Protospacer Adjacent Motif; HR: Homologous Recombination; NHEJ: Non-Homologous End Joining; DMD: Duchenne Muscular Dystrophy.

### History of Gene Editing

A new tool in gene editing technology can be used to introduce targeted modifications into the

genome. Recently, there are well-defined technologies for gene editing: Transcription Activator-Like Effector Nucleases (TALENs), Zinc Finger Nucleases (ZFNs), and Clustered Regularly Interspaced Palindromic Repeats (CRISPRs) with CRISPR-associated (Cas) nucleases. Each of these systems is characterized by nuclease domain that creates a double-strand cleavage an adaptable sequence-specific DNA binding domain. The site specific DNA binding domains of the ZFN and TALEN systems are based on protein chimeric, whereas the CRISPR-Cas system utilizes an RNA molecule.

The first generation of engineered nucleases for genome editing is ZFN technology.

Zinc Finger amide (ZFP) coalesced to the cleavage domain of the FokI restriction endonuclease. The designed desoxyribonucleic acid binding super molecule domain specifically localizes the ZFN at a present location within the genomic sequence of interest and facilitates targeted order written material by making double-strand cleavage by the catalytic domain of the FokI endonuclease.

TALENs, the second generation of ordering engineering nucleases, were discovered in 2010[3] and gained fast momentum among researchers as a result of these enzyme molecules allow a lot of foreseeable and specific binding to target DNA[4]. A TALEN has 2 practical domains: a transcription activator-like effector domain, that is associate oligopeptide array of modules (each module constitutes 33~35 amino acids) from the microorganism bacteria genus spp., and a cleavage domain of FokI nuclease.

CRISPR together with Cas proteins form the CRISPR-Cas system[8], which is the newest gene editing method. CRISPRs represent a family of short desoxyribonucleic acid repeats that ar vital parts of the adjustive system in microorganism and archaea.

These components defend the microbes against varied microorganism invasions.

Cas proteins have useful domains that ar almost like nucleases, helicases, polymerases, and polynucleotide-binding proteins[9].

Basically, the CRISPR-Cas system divided into eight subtypes[9].

However, a replacement system is introduced as a result of the previous classification didn't take into thought the distant relationships between numerous Cas proteins[10]. In this new classification, the CRISPR-Cas system is split into 3 differing types. The type I and III systems involve the specialized Cas endonucleases that method the pre-crRNAs and once mature, the crRNA can assemble into an oversized Cas macromolecule complicated. The complex is capable to recognize and cleave nucleic acids complementary to the crRNA[11].

The type II, that discussion here, CRISPR-Cas9 system is characterized as a little RNA-based system of microorganism and archaea[8], and recently it fully was developed for economical order engineering[12,13]. This technique is straightforward to style, extremely specific, efficient, and well-suited for high- outturn and multiplexed cistron piece of writing for a spread of cell varieties and organisms[15].

An built single guide ribonucleic acid (sgRNA) containing a CRISPR ribonucleic acid (crRNA) and a part complementary trans-activating ribonucleic acid (tracrRNA) square measure necessary and spare for ordering redaction[12]. The primary twenty nucleotides set at the 5'-end of sgRNA, referred to as the spacer, direct the Cas9 enzyme to the complementary twenty nucleotides of the target sequence, wherever they interbreed. A extremely preserved secondary structure downstream of the spacer, referred to as the protospacer, within the presence of a brief ester motif (known because the Protospacer Adjacent Motif (PAM)) (Figure No.1) should be recognized by the CRISPRCas9 enzyme complicated before cleavages happens[13-16]. The CRISPRCas9 system's potential to focus on genomes seems to need solely a PAM sequence (NGG). This simplicity makes it a innovative ordering redaction tool.

**Processes in Genome Editing**

Developing a ordination redaction tool needs engineering endonucleases that may produce extremely economical and correct DSBs at a user

outlined location within the ordination and later on activate the cellular pathways involved in DSB repair processes via Homologous Recombination (HR)-mediated cistron repair or Non- Homologous finish connexion (NHEJ).

HR uses homologous DNA sequences as templates for precise repair. It involves strand invasion and needs a homologous DNA templet to exactly edit a genomic sequence or insert exogenous DNA that ends up in cistron knock out or cistron knock in.

NHEJ is AN erring ligature method that ends up in little insertions or deletions (indel mutations).

This process involves the re-ligation of the two broken ends at the cleavage sites and is catalyzed by DNA ligases[16,17]. Indel generation is exploited as a convenient method for gene silencing (knock-out mutation).

CRISPR is capable of modifying the body target by indel mutations at high frequency[18].

In addition, CRISPR-Cas9 permits the concurrent targeting of many sequences for multiplexed cistron redaction[14,19] and has the potential for cistron replacement by at the same time targeting the sequences upstream and downstream from a given locus[20].

The CRISPR-Cas system is a prokaryotic immune system. The type II CRISPR-Cas9 system is wide used for economical ordination redaction[14,19,21-23] and therefore the institution of factor silencing[24].

When in complicated with sgRNA (Figure No.1), Cas9 introduces DSBs in an exceedingly target sequence that's homologous to the spacer moiety of crRNA[11].

The generation of DSBs within the target polymer initiates the ordination redaction method.

Chromosomal DSBs trigger polymer repair either by unit of time or NHEJ within the absence of a homologous repair templet (Figure No.2). These repair systems is controlled for ordination redaction[25].

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**Genome-scale CRISPR knock-out screening**

Recent progress in modifying CRISPR has junction rectifier to terribly economical sequence disruption in several model organisms[12,19,26-28]. For example, the cistronration of gene secret writing amino acid essential amino acid enzyme ROP[18] knock outs in the type 1 GT1 strain of Toxoplasma gondii using CRISPRCas9 extends reverse genetic techniques to diverse isolates of T. gondii[29].

Chen *et al.* initially incontestable that DSBs is also designed at precise locations inside the Caenorhabditis elegans ordination by injecting the CRISPR-Cas9 difficult, resulting in gene knock out[30].

DSBs can also be used for transgene- educated sequence conversion and permit for the systematic study of sequence perform during this wide used model organism[30].

High rates of mutagenesis efficiency (75-99%) have been reported when testing one homozygous egfp reporter gene and four endogenous loci in zebra fish[31].

These five genomic loci is also targeted at identical time, resulting in multiple loss-of-function phenotypes in the same injected fish, which in turn support multiple biallelic gene inactivations[31].

Similarly, the simultaneous disruption of five genes (Tet1, 2, 3, Sry, Uty-8 alleles) in mouse embryonic stem cells was observed when CRISPR-Cas- mediated gene editing was employed. Further co-injection of Cas9 mRNA and sgRNAs targeting Tet1 and Tet2 into zygotes generated mice with biallelic mutations in both

genes with an efficiency of 80%. This shows that CRISPR-Cas9 is extremely economical at synchronic targeting of multiple genes in stem cells and mice[19].

In another study, the eyeless sequence in branchiopod an magna was knocked out victimisation CRISPR- Cas, suggesting that it's a helpful marker sequence during this system[32]. The CRISPR-Cas9 system also has been used to generate stable knock-out cell line models in human endometrial cell lines[33], human myeloid leukemia cells[34], and human melanoma cells[35] for genetic screening. These findings suggest that the knock-out cell line models generated by the CRISPR-Cas9 system could be used to complement mouse knock-out models, which will offer a new strategy for investigating the function of genes in differentiated cells and tissues.

For example, studies of the disruption of four genes (ApoE, B2m, Prf1, and Prkdc) in rats by co-injection of Cas9 mRNA and sgRNA into one cell fertilized eggs demonstrated the potential of the CRISPR system to expeditiously and faithfully sequencerate gene knock-out rats[36]. Furthermore, RNA guided endonucleases (RGENs) containing Cas9 protein and sgRNA complexes efficiently induced mutations in the mouse Prkdc gene in up to 93% of newborn mice with minimal toxicity[37], that suggests CRISPR-Cas9-mediated cause in animals model has been achieved.

Genome-scale CRISPR knock-in screening

Genome-scale CRISPR knock-in screening

Knock-in ways think about unit of time between built desoxyribonucleic acid and a targeted locus. This approach allows super molecules to be changed at specific loci and to come up with fluorescent macromolecule fusions[30,38].

Homologous repair of Cas9-induced DSBs has been incontestible in multiple organisms[38-40]. For example, CRISPR-Cas9-mediated knock-in of DNA cassettes into the zebra fish genome at predetermined target sites occurred at a very high rate via homology-independent DSB repair pathways[20].

Di Carlo *et al.* demonstrated high frequencies of oligonucleotide recombination with a transient gRNA CRISPR system in yeast[39]. They showed that co-transformation of a gRNA inclusion body and a donor deoxyribonucleic acid in cells resulted in virtually 100 percent donor deoxyribonucleic acid recombination frequency.

In addition, CRISPR-Cas9 achieved knock in to destabilization domain tag the essential gene Treacher Collins-Franceschetti syndrome 1 in human 293T cells, leading to rapid modulation of protein levels in mammalian cells[40].

Gratz *et al.* reported that a fifty ester modification was with success introduced into the yellow locus by Cas9-induced time unit in pomace fly[41] by co-injection of Cas9 template RNA, gRNAs against yellow and fiber oligos as donor DNA for recombination. In another study, Xue *et al.* Used the Cas9 transgenic system to get knock-in mutations in fruit fly by insertion of an oversized piece of two computer memory unit heterologous deoxyribonucleic acid power-assisted by the utilization of a noticeable marker[42].

CRISPR Advanced Gene Therapy

Xie *et al.* used CRISPR-Cas ordering piece of writing technology to correct disease-causing mutations in cells from beta-thalassemia patients; the correction in human elicited pluripotent stem cells fixed traditional perform and provided an upscale source of cells for transplantation[43]. Ebina *et al.* reported that CRISPR geared toward disrupting HIV-1 provirus could also be capable of eradicating infectious agent orderings from infected people by piece of writing the HIV-1 genome and interference its expression.

They found that the CRISPR- Cas9 system is in a position to get rid of internal infectious agent genes from the host cell body[44]. Zhen *et al.* recently incontestible that the CRISPR-Cas9 targeting promoter of human papillomavirus oncogenes (E6 and E7) resulted in accumulation of p53 and p21 proteins and markedly suppressed the proliferation of cervical cancer cells in vitro and *in vivo*[45]. In another study, a dominant cataract-causing mutation in the Crygc gene in mice was corrected using CRISPR-Cas9, thereby demonstrating the potential of this system for

efficient correction of a genetic disease[46]. The CRISPR-Cas9 ordering writing system additionally has the potential to repair the pancreatic fibrosistrans membrane conductor receptor locus by time unit in civilized enteral stem cells of pancreatic fibrosis patients[47].

Long *et al.*[48] recently used the CRISPR-Cas9 issue orthography to correct the dystrophin mutation in developing mdx mice; a model for in Duchenne genetic disorder (DMD) suggests that CRISPR-Cas9 factor therapy might work in animals.

The corrected cells could ultimately generate several healthy muscle fibers wherever this strategy could in some unspecified time {in the future} enable correction of disease-causing mutations in the muscle tissue of patients with doctorate[48].

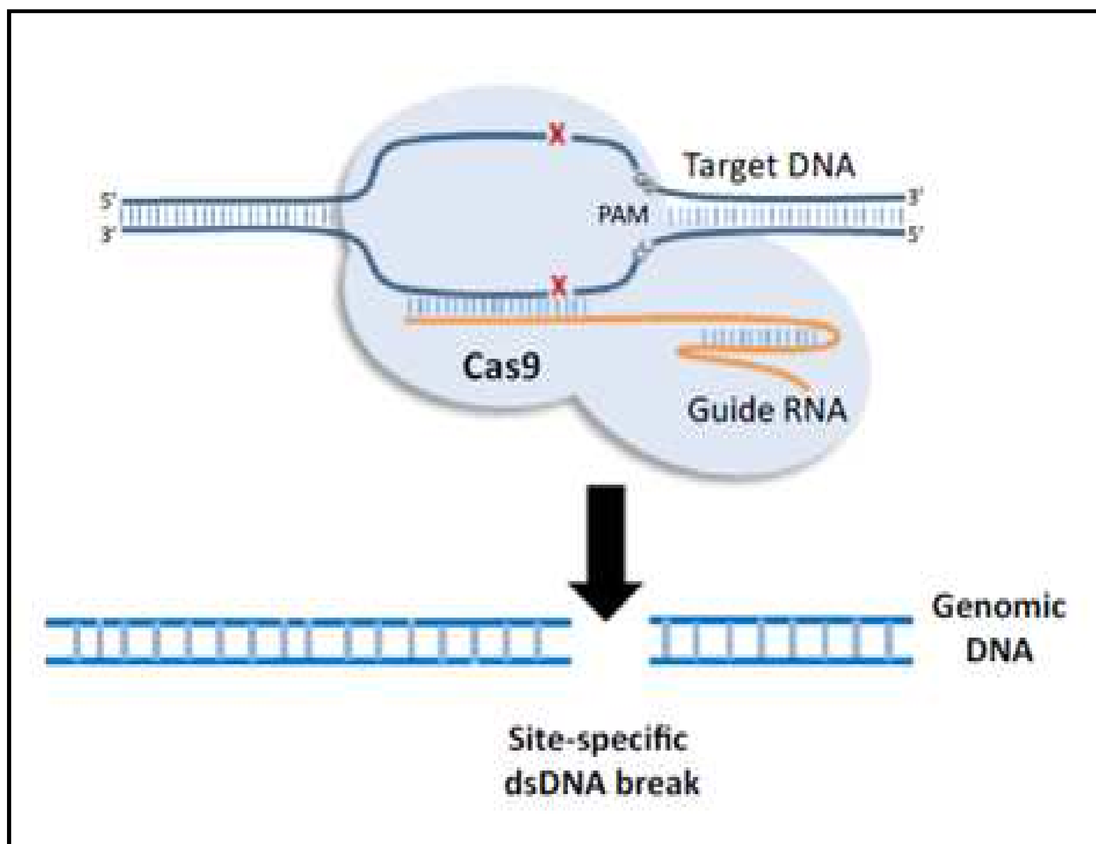


**Figure No.1: CRISPR-Cas9 target recognition. In the CRISPR system, a sgRNA (containing a CRISPR RNA (crRNA) and a partially complementary trans activating RNA (tracrRNA) is essential for RNA processing and for recognition by Cas9 (CRISPR- associated protein 9). Cas9 is a RNA-guided, dsDNA binding protein that uses a nuclease to cleave both strands of target DNA. Cas9 relies on the PAM site and base pairing with the sgRNA and the target DNA**

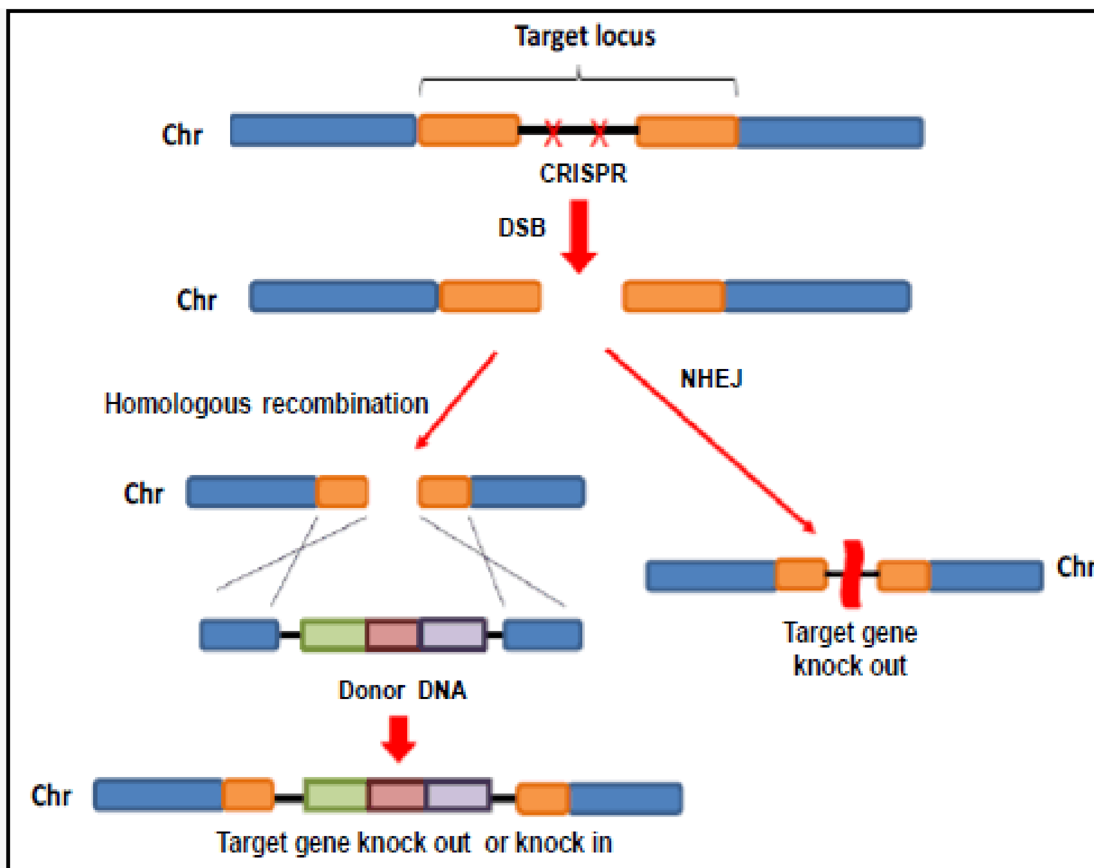


**Figure No.2: Genomic double stranded DNA breaks (DSBs) are repaired by Homologous Recombination (HR) or Non-Homologous End Joining (NHEJ). HR relies on a donor template that can be used to deliver foreign DNA at a specific location. NHEJ is prone to errors, resulting in indel mutations (insertions or deletions) that disrupt the target site**

## CONCLUDING REMARKS AND FUTURE DIRECTIONS

The simplicity of programming CRISPR-Cas9 has contributed to its speedy implementation in ordering engineering. Its use has resulted in rapid generation of genome-scale knock-out libraries for complex model systems, including human cells and animal disease models, and it has potential for use in ex vivo gene therapy in humans. Nevertheless, the CRISPR system may have limitations that have yet to be identified. The delivery and specificity of the gRNA remains not absolutely understood. Hence, the effectiveness of the CRISPR delivery system should be measured and any valid so as to utilize this technique as a secure and reliable tool, particularly within the treatment of human diseases.


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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

## BIBLIOGRAPHY


1. Wolfe S A, Nekludova L, Pabo C O. DNA recognition by Cys2His2 zinc finger proteins, *Annu Rev Biophys Biomol Struct,* 29, 2000, 183-212.
2. Christian M, Cermak T, Doyle E L, Schmidt C, Zhang F, *et al.* Targeting DNA double-

strand breaks with TAL effector nucleases, *Genetics,* 186(2), 2010, 757-761.

3. Boch J, Bonas U. Xanthomonas AvrBs3 family-type III effectors: discovery and function, *Annu Rev Phytopathol,* 48, 2010, 419-436.
4. Beumer K J, Trautman J K, Christian M, Dahlem T J, Lake C M, *et al.* Comparing zinc finger nucleases and transcription activator-like effector nucleases for gene targeting in Drosophila, *G3 (Bethesda),* 3(9), 2013, 1717-1725.
5. Boch J, Scholze H, Schornack S, Landgraf A, Hahn S, *et al.* Breaking the code of DNA binding specificity of TAL-type III effectors, *Science,* 326(5959), 2009, 1509-1512.
6. Meng X, Noyes M B, Zhu L J, Lawson N D, Wolfe S A. Targeted gene inactivation in zebrafish using engineered zinc-finger nucleases, *Nat Biotechnol,* 26(6), 2008, 695-701.
7. Horvath P, Barrangou R. CRISPR/Cas, the immune system of bacteria and archaea, *Science,* 327(5962), 2010, 167-170.
8. Haft D H, Selengut J, Mongodin EF, Nelson K E. A guild of 45 CRISPR-associated (Cas) protein families and multiple CRISPR/Cas subtypes exist in prokaryotic genomes, *PLoS Comput Biol,* 1(6), 2005, e60.
9. Van Der Oost J, Jore M M, Westra E R, Lundgren M, Brouns S J. CRISPR-based adaptive and heritable immunity in prokaryotes, *Trends Biochem Sci,* 34(8), 2009, 401-407.
10. Makarova K S, Haft D H, Barrangou R, Brouns S J, Charpentier E, *et al.* Evolution and classification of the CRISPR-Cas systems, *Nat Rev Microbiol,* 9(6), 2011, 467-477.
11. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna J A, *et al.* A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity, *Science,* 337(6096), 2012, 816-821.
12. Mali P, Yang L, Esvelt K M, Aach J, Guell M, *et al.* RNA-guided human genome engineering via Cas9, *Science,* 339(6121), 2013, 823-826.
13. Hwang W Y, Fu Y, Reyon D, Maeder M L, Tsai S Q, *et al.* Efficient genome editing in zebrafish using a CRISPR-Cas system, *Nat Biotechnol,* 31(3), 2013, 227-229.
14. Cong L, Ran F A, Cox D, Lin S, Barretto R, *et al.* Multiplex genome engineering using CRISPR/Cas systems, *Science,* 339(6121), 2013, 819-823.
15. Ran F A, Hsu P D, Wright J, Agarwala V, Scott D A, *et al.* Genome engineering using the CRISPR-Cas9 system, Nat Protoc, 8(11), 2013, 2281-2308.
16. Bassing C H, Alt F W. The cellular response to general and programmed DNA double strand breaks, *DNA Repair (Amst),* 3(8-9), 2004, 781-796.
17. Gratz S J, Ukken F P, Rubinstein C D, Thiede G, Donohue L K, *et al.* Highly specific and efficient CRISPR/Cas9-catalyzed homology-directed repair in Drosophila, *Genetics,* 196(4), 2014, 961-971.
18. Cradick T J, Fine E J, Antico C J, Bao G. CRISPR/Cas9 systems targeting Î²-globin and CCR5 genes have substantial off-target activity, *Nucleic Acids Res,* 41(20), 2013, 9584-9592.
19. Wang H, Yang H, Shivalila C S, Dawlaty M M, Cheng A W, *et al.* One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering, *Cell,* 153(4), 2013, 910-918.
20. Auer T O, Duroure K, De Cian A, Concordet J P, Del Bene F. Highly efficient CRISPR/Cas9-mediated knock-in in zebrafish by homology-independent DNA repair, *Genome Res,* 24(1), 2014, 142-153.
21. Richter H, Randau L, Plagens A. Exploiting CRISPR/Cas: interference mechanisms and applications, *Int J Mol Sci,* 14(7), 2013, 14518-14531.
22. Cho S W, Kim S, Kim J M, Kim J S. Targeted genome engineering in human cells with the Cas9 RNA-guided

endonuclease, *Nat Biotechnol,* 31(3), 2013, 230-232.

23. Jiang W, Bikard D, Cox D, Zhang F, Marraffini L A. RNA-guided editing of bacterial genomes using CRISPR-Cas systems, *Nat Biotechnol,* 31(3), 2013, 233-239.
24. Qi LS, Larson M H, Gilbert L A, Doudna J A, Weissman J S, *et al.* Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression, *Cell,* 152(5), 2013, 1173-1183.
25. Allen C, Kurimasa A, Brenneman M A, Chen D J, Nickoloff J A. DNA-dependent protein kinase suppresses double-strand break-induced and spontaneous homologous recombination, *Proc Natl Acad Sci U S A* 99(6), 2002, 3758-3763.
26. Ren X, Sun J, Housden B E, Hu Y, Roesel C, *et al.* Optimized gene editing technology for Drosophila melanogaster using germ line-specific Cas9, *Proc Natl Acad Sci U S A,* 110(47), 2013, 19012-19017.
27. Hruscha A, Krawitz P, Rechenberg A, Heinrich V, Hecht J, *et al.* Efficient CRISPR/Cas9 genome editing with low off-target effects in zebrafish, *Development,* 140(24), 2013, 4982-4987.
28. Friedland A E, Tzur Y B, Esvelt K M, Colaiacovo M P, Church G M, *et al.* Heritable genome editing in C. elegans via a CRISPR-Cas9 system, *Nat Methods,* 10(8), 2013, 741-743.
29. Shen B, Brown K M, Lee T D, Sibley LD. Efficient gene disruption in diverse strains of Toxoplasma gondii using CRISPR/CAS9, *MBio,* 5(3), 2014, e01114-01114.
30. Chen C, Fenk L A, De Bono M. Efficient genome editing in Caenorhabditis elegans by CRISPR-targeted homologous recombination, *Nucleic Acids Res,* 41(20), 2013, e193.
31. Jao L E, Wente S R, Chen W. Efficient multiplex biallelic zebrafish genome editing using a CRISPR nuclease system, *Proc Natl Acad Sci U S A,* 110(34), 2013, 13904-13909.
32. Nakanishi T, Kato Y, Matsuura T, Watanabe H. CRISPR/Cas-mediated targeted mutagenesis in Daphnia magna, *PLoS One,* 9(5), 2014, e98363.
33. Lacroix Pépin N, Chapdelaine P, Rodriguez Y, Tremblay J P, Fortier M A. Generation of human endometrial knockout cell lines with the CRISPR/Cas9 system confirms the prostaglandin F2Î± synthase activity of aldo-ketoreductase 1B1, *Mol Hum Reprod,* 20(7), 2014, 650-663.
34. Wang T, Wei J J, Sabatini D M, Lander E S. Genetic screens in human cells using the CRISPR-Cas9 system, *Science,* 343(6166), 2014, 80-84.
35. Shalem O, Sanjana N E, Hartenian E, Shi X, Scott D A, *et al.* Genome-scale CRISPR-Cas9 knockout screening in human cells, *Science,* 343(6166), 2014, 84-87.
36. Ma Y, Shen B, Zhang X, Lu Y, Chen W, *et al.* Heritable multiplex genetic engineering in rats using CRISPR/Cas9, *PLoS One,* 9(3), 2014, e89413.
37. Sung Y H, Kim J M, Kim H T, Lee J, Jeon J, *et al.* Highly efficient gene knockout in mice and zebrafish with RNA-guided endonucleases, *Genome Res,* 24(1), 2014, 125-131.
38. Dickinson D J, Ward J D, Reiner D J, Goldstein B. Engineering the Caenorhabditis elegans genome using Cas9-triggered homologous recombination, *Nat Methods,* 10(10), 2013, 1028-1034.
39. DiCarlo J E, Norville J E, Mali P, Rios X, Aach J, *et al.* Genome engineering in Saccharomyces cerevisiae using CRISPR-Cas systems, *Nucleic Acids Res,* 41(7), 2013, 4336-4343.
40. Park A, Won S T, Pentecost M, Bartkowski W, Lee B. CRISPR/Cas9 allows efficient and complete knock-in of a destabilization domain-tagged essential protein in a human cell line, allowing rapid knockdown of protein function, *PLoS One,* 9(4), 2014, e95101.

41. Gratz S J, Cummings A M, Nguyen J N, Hamm D C, Donohue L K, *et al.* Genome engineering of Drosophila with the CRISPR RNA-guided Cas9 nuclease, *Genetics,* 194(4), 2013, 1029-1035.
42. Xue Z, Ren M, Wu M, Dai J, Rong Y S, *et al.* Efficient gene knock-out and knock-in with transgenic Cas9 in Drosophila, *G3 (Bethesda),* 4(4), 2014, 925-929.
43. Xie F, Ye L, Chang J C, Beyer A I, Wang J, *et al.* Seamless gene correction of Î²-thalassemia mutations in patient-specific iPSCs using CRISPR/Cas9 and piggyBac, Genome Res, 24(9), 2014, 1526-1533.
44. Ebina H, Misawa N, Kanemura Y, Koyanagi Y. Harnessing the CRISPR/Cas9 system to disrupt latent HIV-1 provirus, *Sci Rep,* 3, 2013, 2510.
45. Zhen S, Hua L, Takahashi Y, Narita S, Liu Y H, *et al. In vitro* and *in vivo* growth suppression of human papillomavirus 16-positive cervical cancer cells by CRISPR/Cas9, *Biochem Biophys Res Commun,* 450(4), 2014, 1422-1426.
46. Wu Y, Liang D, Wang Y, Bai M, Tang W, *et al.* Correction of a genetic disease in mouse via use of CRISPR-Cas9, *Cell Stem Cell,* 13(6), 2013, 659-662.
47. Schwank G, Koo B K, Sasselli V, Dekkers J F, Heo I, *et al.* Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients, *Cell Stem Cell,* 13(6), 2013, 653-658.
48. Long C, McAnally J R, Shelton J M, Mireault A A, Bassel-Duby R, *et al.* Prevention of muscular dystrophy in mice by CRISPR/Cas9-mediated editing of germline DNA, *Science,* 345(6201), 2014, 1184-1188.

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