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Gene editing: Revolution for genome modification and its scope in healthcare research

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Abstract
Genome editing is a technique that has opened the door for a new concept in which we can manipulate the genome sequence for different purposes. Generation of specific pre-planned changes for genome manipulation includes double-stranded breaks. The double-stranded breaks use the intrinsic repair mechanism of the cell. Four major methods for inducing site-specific double-stranded breaks which are CRISPR, TALENs, ZFN, and Mega nuclease. Out of these CRISPR is the most recent and easy-to-use technique for genome editing. It has provided powerful tools for the precise modification of genetic material. Genome editing has a wide range of purposes in different sectors including healthcare, therapeutics, agriculture, etc.

Keywords: Genome editing, CRISPR Cas9, TALENs, ZFN, Human health

Introduction
The addition of new genes to the cells is known as gene therapy which has been used for ages. Recently, gene editing techniques have opened the door to a new concept in which the exact manipulation of genome sequences for different purposes is now possible (Maeder & Gersbach, 2016) [18]. The field of biotechnology has witnessed a revolution with the development of 'engineered' and/or 'programmable' enzymes for the modification of DNA sequences, despite the extensive research and interest in gene therapy and genome editing over the years (Doudna, 2020) [7]. Recently different platforms have been discovered for genetically modifying somatic and pluripotent stem cells. These comprise 'CRISPR (Clustered Regularly Interspersed Short Palindromic Repeats)', 'TALENs (Transcription Activator-like Effector Nucleases)', and 'ZFN (zinc finger nucleases)'. CRISPR/Cas is a combination of CRISPR and the protein CRISPR-associated protein (Broeders *et al*., 2020 Rodríguez-Rodríguez *et al*., 2019) [5, 23]. Generating specific, pre-planned changes in the genome is a prerequisite for genome editing which includes double-strand break, single-strand break, or certain base alteration which result in the activation of endogenous repairing process which further allows for the modification of the genome (Broeders *et al*., 2020) [5]. This review presents an insights on the potential impact of gene-editing technologies in the area of healthcare.

Mechanism of editing genomes
The understanding that specific DNA double-stranded breaks can trigger the inherent cellular repair mechanism within the body served as the basis for genome editing. These breaks are typically repaired by one of the two main pathways that are Homology Directed Repair (HDR) and Non-homologous End Joining (NHEJ) (Takata *et al*., 1998) [27].
Homology Directed Repair relies on strand invasion and template-dependent repair (Szostak *et al*., 1983) [26]. Double-stranded breaks enhance gene targeting efficiency in mammalian cells (Maeder & Gersbach, 2016) [18]. In contrast to HDR, Non-Homologous End Joining uses direct ligation of cleaved ends to repair DSBs without the need of a template (Lieber *et al*., 2003) [16]. Due to its error-prone nature, this mechanism frequently leads to indels (insertion or deletions) of genetic material at the site of the break. The ability to modify these indels and altering the reading frame of a gene has been utilized to disrupt target gene in various cell types and organisms by stimulating Non-Homologous End Joining through site-specific DNA double stranded breaks.

Nowadays it has become possible to achieve a wide range of specific genomic modifications by using the capacity of the cell's intrinsic DNA repair machinery (Maeder & Gersbach, 2016) [18].

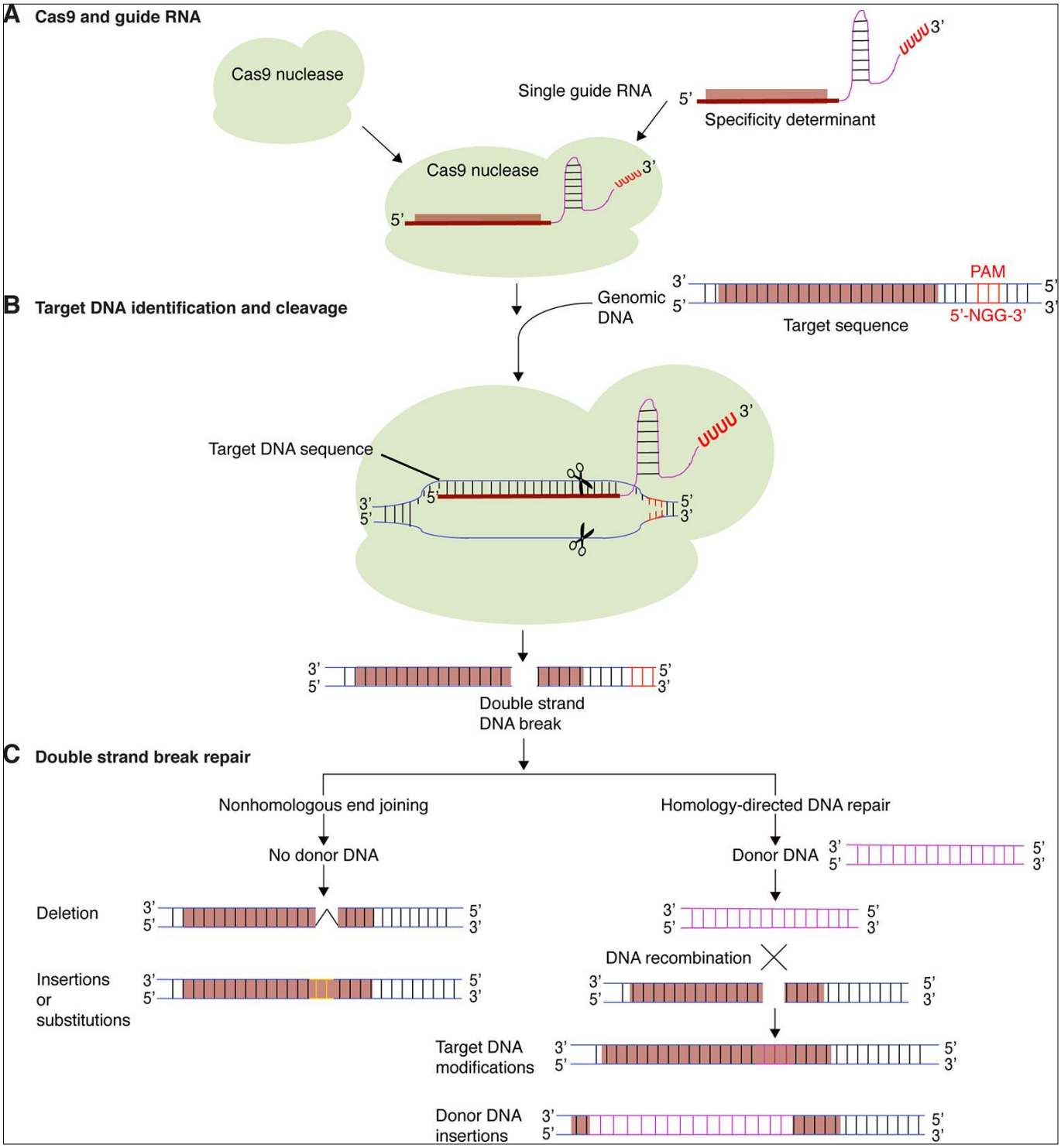

Fig 1: Mechanisms of double-strand break repair (Figure adapted from El-Mounadi *et al*., 2020) [8]

CRISPR

CRISPR technology, derived from the defense system of certain bacteria against viruses and plasmids, has sparked a renaissance in genome editing since its emergence in 2012. The CRISPR-Cas9 system harnesses the Cas9 nuclease, guided by a short RNA molecule, to precisely create site-specific double-stranded DNA breaks. This versatile and user-friendly method has undergone extensive testing across a range of experimental models, including cell lines, laboratory animals, plants, and even human clinical trials. Its broad application has accelerated basic research and facilitated advancements in agriculture and synthetic biology. Recent studies have unveiled the remarkable role of CRISPR and CRISPR-associated (Cas) proteins in providing microorganisms with adaptive immunity, paving the way for ground-breaking technological advancements guided by RNA (Knott & Doudna, 2018) [14]. Cas9 assembles with the "guide RNA" to form a biochemical unit capable of binding and cutting DNA. This can occur with separate "crRNA" and "tracrRNA" components or as a chimeric "single guide RNA" (Jinek *et al*., 2012) [13]. DNA binding occurs when a 20-base pair DNA sequence is complementary to a corresponding

sequence in the guide RNA, which can be easily customized by researchers (Gasiunas *et al*., 2012; Jinek *et al*., 2012) [10, 13]. A short motif acting as a switch must be located near the DNA recognition site for Cas9 to cleave the target sequence's double-stranded DNA (Gasiunas *et al*., 2012; Jinek *et al*., n.d.) [10, 13]. These double-stranded DNA breaks stimulate inherent cellular processes for DNA repair, introducing sequence variations such as minor changes or the insertion of genetic material (Stark *et al*., 2004; Szostak *et al*., 1983) [25, 26]. Mammalian cells with double-stranded DNA breaks undergo DNA repair, leading to site-specific changes in genome sequences. The Cas9 enzyme, derived from Streptococcus pyogenes, is widely utilized for genome editing and genetic manipulation using the CRISPR-Cas system (Liu *et al*., 2019) [17]. CRISPR-Cas-induced DNA cleavage triggers genome editing through the repair of double-stranded DNA breaks via non-homologous end joining (NHEJ) or homology-directed repair (HDR). In addition to DNA cleavage-based editing, CRISPR-Cas9 enables direct modification of the chemical sequence, known as base editing (Doudna, 2020; Komor *et al*., 2016; Nishida *et al*., 2016) [7, 15, 21]. Another approach, prime editing, utilizes RNA templates for precise gene alterations (Anzalone *et al*., 2019; Doudna, 2020; Sharon *et al*., 2018) [3, 7, 24]. Moreover, CRISPR interference and CRISPR activation provide powerful tools for transcriptional control (Doudna, 2020; Gilbert *et al*., 2014; Qi *et al*., 2013) [7, 11, 22].

Application of genome editing technology for human health

The field of genome editing holds great promise in preventing viral infections and their replication. An advanced technique involves modifying T-cells ex vivo to disable the CCR5 coreceptor, which plays a crucial role in initial HIV infection (Holt *et al*., 2010) [12]. Beyond HIV, genome editing platforms are used to target various other viral pathogens like hepatitis B virus, herpes simplex virus, and human papillomavirus. The approach entails the precise targeting and elimination of viral genomes, with a primary emphasis on essential genes that contribute to the stability and replication of the genome (Maeder & Gersbach, 2016) [18]. In recent times, gene editing techniques have emerged as promising instruments for rectifying gene mutations associated with specific immune disorders. Precisely, conditions like ADA-SCID and radiosensitive SCID, resulting from reduced DNA-dependent Protein kinase activity, have been targeted for correction (Maeder & Gersbach, 2016) [18]. Within the domain of liver diseases, targeted gene repair holds immense potential in addressing clotting disorders, such as haemophilia ‘A’ and ‘B’. Furthermore, it presents encouraging possibilities for treating lysosomal storage disorders, encompassing 'Fabry disease', 'Gaucher disease', 'Hurler and Hunter syndromes', 'Pompe disease', and 'von Gierke disease' (Maeder & Gersbach, 2016) [18]. Additionally, strategic manipulation of specific genes in the liver can yield favorable outcomes. An exemplification of this lies in the ‘PCSK9 gene’, which encodes a proteinase responsible for degrading the low-density lipoprotein receptor (LDLR). Diminished LDLR levels impede the metabolism of ‘LDL-C’, thereby heightening the susceptibility to cardiovascular ailment. The exploration of natural genetic variations influencing ‘PCSK9’ activation and cholesterol levels has ignited substantial interest within the scientific community. As a result, the development of PCSK9-blocking drugs aimed at diminishing cholesterol levels has garnered noteworthy attention (Abifadel *et al*., 2003, 2014) [1, 2]. Significant advancements have been achieved in the field of gene delivery and cell transplantation, providing exciting prospects for gene and cell therapy in a range of neuromuscular disorders. These include ‘Duchenne Muscular Dystrophy’, ‘limb-girdle muscular dystrophies’, ‘spinal muscular atrophy’, ‘Friedreich's ataxia’, ‘Huntington's disease’, and ‘amyotrophic lateral sclerosis’ (Maeder & Gersbach, 2016) [18]. These breakthroughs have paved the way for innovative approaches in treating and managing these debilitating disorders, particularly in the central nervous system, skeletal muscle, and cardiac muscle. The treatment options for genetic skin disorders have expanded with the development of synthetic skin grafts derived from autologous and allogeneic cells, including induced pluripotent stem (iPS) cells. For instance, recessive dystrophic epidermolysis bullosa, characterized by alterations in the gene encoding ‘type VII collagen’, causes severe skin blistering due to impaired type VII collagen expression. By utilizing patient cells to create customized skin grafts and editing their genomes, it becomes possible to treat this disorder (Maeder & Gersbach, 2016) [18]. Cystic fibrosis, resulting from a mutation in the CFTR chloride-channel gene, leads to dysregulated epithelial fluid transport in multiple organs due to the loss of chloride channel function. Gene editing has shown successful correction of CFTR mutations in intestinal stem cells derived from patients and induced pluripotent stem cells with the potential to differentiate into epithelial cells (Crane *et al*., 2015; Firth *et al*., 2015) [6, 9]. However, the efficient delivery of the gene to lung epithelium remains a challenge. The intranasal delivery of nanoparticles (NPs) carrying ‘triplex-forming peptide nucleic acid (PNA)’ has shown promising results in mouse models (McNeer *et al*., 2015) [19]. A notable application of genome editing is its recent progress in tackling pathogenic bacterial infections. Gene editing nucleases can target specific genes associated with virulence or antibiotic resistance, aiming to mitigate their adverse effects (Maeder & Gersbach, 2016) [18].

Scope of gene editing technologies

In biomedical research, genome editing act as a potential technology that offers hope for treating some inherited diseases. Sickle cell anemia and muscular dystrophy are two prevalent human genetic abnormalities that serve as an example of diseases that can be treated or cured by genome editing shortly (Doudna, 2020) [7]. CRISPR-enabled genome editing has been used in research to alter epigenomes, control transcription, running genome-wide screens, and chromosome imaging (Barrangou & Doudna, 2016)[4]. The ability of CRISPR Cas9 for both fundamental and translational research of cancer is being discovered. The majority of cancer cell lines will have a complete collection of critical genes using pooled CRISPR screens in the near future. These along with previously available resources on genetic features of cancer cell lines will greatly contribute to the identification of lethal interactions and enable the discovery of novel drug targets (Zhan *et al*., 2019) [28].

Conclusion

Gene editing is a widely used technology having the potential to revolutionize the agriculture and medicine industry. The field has gone through significant development in recent years, which offers new possibilities for precise genome manipulation with high precision for various purposes. The

arrival of CRISPR technology which is derived from bacteria's defense system has enhanced research and advancement in gene editing. Scientists are able to create new treatments for diseases by precise alteration in the gene. One of the most important applications of genome editing is the treatment of genetic diseases such as the use of genome editing in the treatment of SCID, DMD, and Haemophilia. Gene editing is also helpful in the treatment of HIV and other viral diseases. Further, the use of gene editing in cancer research is under investigation. CRISPR is used in the alteration of epigenomes to control transcription. Despite being a potential technology gene editing is still in its early developmental phase. There are many ethical concerns that need to be addressed before using gene editing on a large scale.

References

1. Abifadel M, Elbitar S, El Khoury P, Ghaleb Y, Chémaly M, Moussalli M-L, *et al*. Living the PCSK9 adventure: from the identification of a new gene in familial hypercholesterolemia towards a potential new class of anticholesterol drugs. Current Atherosclerosis Reports. 2014;16(9):439. https://doi.org/10.1007/s11883-014-0439-8
2. Abifadel M, Varret M, Rabès J-P, Allard D, Ouguerram K, Devillers M, *et al*. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nature Genetics. 2003;34(2):154-156. https://doi.org/10.1038/ng1161
3. Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW, Levy JM, *et al*. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature. 2019;576(7785):149-157. https://doi.org/10.1038/s41586-019-1711-4
4. Barrangou R, Doudna JA. Applications of CRISPR technologies in research and beyond. Nature Biotechnology. 2016;34(9):933-941. https://doi.org/10.1038/nbt.3659
5. Broeders M, Herrero-Hernandez P, Ernst MPT, van der Ploeg AT, Pijnappel WWMP. Sharpening the Molecular Scissors: Advances in Gene-Editing Technology. IScience. 2020;23(1):100789. https://doi.org/10.1016/J.ISCI.2019.100789
6. Crane AM, Kramer P, Bui JH, Chung WJ, Li XS, Gonzalez-Garay ML, *et al*. Targeted correction and restored function of the CFTR gene in cystic fibrosis induced pluripotent stem cells. Stem Cell Reports. 2015;4(4):569-577. https://doi.org/10.1016/j.stemcr.2015.02.005
7. Doudna JA. The promise and challenge of therapeutic genome editing. Nature. 2020;578(7794):229-236. https://doi.org/10.1038/s41586-020-1978-5
8. El-Mounadi K, Morales-Floriano ML, Garcia-Ruiz H. Principles, applications, and biosafety of plant genome editing using CRISPR-Cas9. Frontiers in Plant Science. 2020;11:56. https://doi.org/10.3389/fpls.2020.00056
9. Firth AL, Menon T, Parker GS, Qualls SJ, Lewis BM, Ke E, *et al*. Functional Gene Correction for Cystic Fibrosis in Lung Epithelial Cells Generated from Patient iPSCs. Cell Reports. 2015;12(9):1385-1390. https://doi.org/10.1016/j.celrep.2015.07.062
10. Gasiunas G, Barrangou R, Horvath P, Siksnys V. Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(39):E2579-86. https://doi.org/10.1073/pnas.1208507109
11. Gilbert LA, Horlbeck MA, Adamson B, Villalta JE, Chen Y, Whitehead EH, *et al*. Genome-Scale CRISPR-Mediated Control of Gene Repression and Activation. Cell. 2014;159(3):647-661. https://doi.org/10.1016/j.cell.2014.09.029
12. Holt N, Wang J, Kim K, Friedman G, Wang X, Taupin V, *et al*. Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to CCR5 control HIV-1 *in vivo*. Nature Biotechnology. 2010;28(8):839-847. https://doi.org/10.1038/nbt.1663
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 (New York, N.Y.). 2012;337(6096):816-821. https://doi.org/10.1126/science.1225829
14. Knott GJ, Doudna JA. CRISPR-Cas guides the future of genetic engineering. Science (New York, N.Y.), 2018;361(6405):866-869. https://doi.org/10.1126/science.aat5011
15. 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;533:420-424. https://doi.org/10.1038/nature17946
16. Lieber MR, Ma Y, Pannicke U, Schwarz K. Mechanism and regulation of human non-homologous DNA end-joining. Nature Reviews. Molecular Cell Biology. 2003;4(9):712-720. https://doi.org/10.1038/nrm1202
17. Liu JJ, Orlova N, Oakes BL, Ma E, Spinner HB, Baney KLM, *et al*. CasX enzymes comprise a distinct family of RNA-guided genome editors. Nature. 2019;566(7743):218-223. https://doi.org/10.1038/s41586-019-0908-x
18. Maeder ML, Gersbach CA. Genome-editing technologies for gene and cell therapy. Molecular Therapy: The Journal of the American Society of Gene Therapy. 2016;24(3):430-446. https://doi.org/10.1038/mt.2016.10
19. McNeer NA, Anandalingam K, Fields RJ, Caputo C, Kopic S, Gupta A, *et al*. Nanoparticles that deliver triplex-forming peptide nucleic acid molecules correct F508del CFTR in airway epithelium. Nature Communications, 2015, 6. https://doi.org/10.1038/ncomms7952
20. Montecillo JAV, Chu LL, Bae H. CRISPR-Cas9 system for plant genome editing: Current approaches and emerging developments. Agronomy (Basel, Switzerland). 2020;10(7):1033. https://doi.org/10.3390/agronomy10071033
21. 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(6305). https://doi.org/10.1126/science.aaf8729
22. Qi LS, Larson MH, Gilbert LA, Doudna JA, Weissman JS, Arkin AP, *et al*. Repurposing CRISPR as an RNA-γuided platform for sequence-specific control of gene expression. Cell. 2013;152(5):1173-1183. https://doi.org/10.1016/j.cell.2013.02.022
23. Rodríguez-Rodríguez DR, Ramírez-Solís R, Garza-Elizondo MA, Garza-Rodríguez MDL, Barrera-Saldaña HA. Genome editing: A perspective on the application of CRISPR/Cas9 to study human diseases (Review).

International Journal of Molecular Medicine. 2019;43(4):1559-1574. https://doi.org/10.3892/ijmm.2019.4112
24. Sharon E, Chen SAA, Khosla NM, Smith JD, Pritchard JK, Fraser HB. Functional Genetic Variants Revealed by Massively Parallel Precise Genome Editing. Cell. 2018;175(2):544-557.e16. https://doi.org/10.1016/j.cell.2018.08.057
25. Stark JM, Pierce AJ, Oh J, Pastink A, Jasin M. Genetic Steps of Mammalian Homologous Repair with Distinct Mutagenic Consequences. Molecular and Cellular Biology. 2004;24(21):9305-9316. https://doi.org/10.1128/mcb.24.21.9305-9316.2004
26. Szostak JW, Orr-Weaver TL, Rothstein RJ, Stahl FW. The double-strand-break repair model for recombination. Cell. 1983;33(1):25-35. https://doi.org/10.1016/0092-8674(83)90331-8
27. Takata M, Sasaki MS, Sonoda E, Morrison C, Hashimoto M, Utsumi H, *et al*. Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. EMBO Journal. 1998;17(18):5497-5508. https://doi.org/10.1093/emboj/17.18.5497
28. Zhan T, Rindtorff N, Betge J, Ebert MP, Boutros M. CRISPR/Cas9 for cancer research and therapy. Seminars in Cancer Biology. 2019;55:106-119. https://doi.org/10.1016/j.semcancer.2018.04.001