## **Bacterial-Artificial-Chromosome-Based Genome Editing in Herpesvirus**

#### Subjects: Biochemistry & Molecular Biology

Contributor: Mengling Hao, Jiabao Tang, Shengxiang Ge, Tingdong Li, Ningshao Xia

Herpesviruses are major pathogens that infect humans and animals. Manipulating the large genome is critical for exploring the function of specific genes and studying the pathogenesis of herpesviruses and developing novel antiviral vaccines and therapeutics. Bacterial artificial chromosome (BAC) technology significantly advanced the capacity of herpesviruses researchers to manipulate the virus genomes.

herpesvirus

bacterial artificial chromosomes gene editing virus mutant selection

## 1. Introduction

Genome manipulation is an effective method for studying the function of viral genes and can help scientists understand the biology of viruses, such as discovering virulence factors and exploring new targets for developing novel vaccines and antiviral drugs for the prevention and treatment of viral infection. Herpesviruses are a group of double-strand DNA (dsDNA) viruses which can cause lifelong persistent infections and are major pathogens in humans and a wide range of animals. The genomes of herpesviruses are between 125 and 295 kilobases in length <sup>[1]</sup>, which makes it difficult for genome manipulation.

In the past decades, scientists have developed several methods for manipulating the genome of herpesviruses, such as  $\lambda$ -red, Cre-loxp, CRISPR-Cas9, and others, which helped understand the pathogenesis of herpesviruses and develop novel vaccines and antiviral drugs <sup>[2][3]</sup>. Traditionally, linear DNA fragments or circular plasmids containing selection cassettes and flanking homologous regions were transferred into herpesvirus-infected cells, and the genomes of herpesviruses were edited by recombination, and then the recombinant viruses were selected by using different markers (different selection cassettes) [4][5][6][7]. This approach has a low efficiency. In addition, its dependence on the production of progeny viruses results in a limited range of genes that can be edited. Moreover, the cumbersome screening process for identifying functional recombinant viruses restricts its application 3. Therefore, it is imperative to explore alternative techniques that can enhance the efficiency and expand the range of genes that can be edited. Bacterial artificial chromosomes (BAC), constructed based on factor F of Escherichia coli (E. coli), provide an important technical platform for the editing of large genomes, including herpesviruses [8] [10][11]. BAC-based gene editing strategies are independent of the production of the progeny, and the screening efficiency is higher than conventional strategies [12][13][14]. Firstly, BACs can accommodate the insertion and stable inheritance of exogenous gene fragments ranging from 100 to 300 kb. The F factor facilitates the maintenance of low copy numbers (1-2 copies per cell) in *E. coli* which greatly reduces the difficulty in screening. Meanwhile, the development of various gene editing techniques in E. coli has greatly enhanced the editing efficiency of

herpesvirus BACs compared to traditional methods. Edited BACs can be reintroduced into eukaryotic cells to reconstruct herpesvirus mutants for further study of their biological behaviors in cells. In addition, the selection of recombinantly engineered viruses is not dependent on the generation of progeny viruses, allowing editing of the y herpes virus genome that maintains latent infection in the long term <sup>[13][15]</sup> as well as manipulating genes essential for the production of progeny viruses <sup>[11]</sup>.

# 2. Genome Editing Techniques of Herpesviruses Based on BAC

#### 2.1. RecA Recombination Technique

The RecA recombination technique is a bacterial endogenous homologous recombination system composed of RecA recombinase and related auxiliary proteins (such as RecBCD, RecFOR, RuvABC, etc.). RecBCD is a multifunctional enzyme complex consisting of RecB, RecC, and RecD. When binding to the incision on the double-stranded donor DNA, RecB and RecD exhibit 3' to 5' and 5' to 3' helicase activities, respectively, which together result in the opening of the complementary double-stranded DNA (**Figure 1**a) <sup>[16]</sup>. Then, RecC can mediate the recognition of chi sites in the sequence and thereby cause allosteric changes, leading to a decrease in the pace of movement (**Figure 1**b) <sup>[17]</sup>. At this point, RecB also has exonuclease activity, which mediates the generation of single-stranded DNA at the 3' end (**Figure 1**c) <sup>[18][19][20]</sup>. The RecA recombinase binds to single-stranded DNA sites generated by the exonucleases and engages in the process of identifying homologous regions on the BAC for subsequent annealing and interaction. After successful interaction, the nucleoprotein filaments invade the dsDNA and undergo strand exchange to form heteroduplex DNA (**Figure 1**d). Finally, RuvABC assists in catalyzing branch migration and degradation of the heteroduplex DNA, resulting in homologous recombination (**Figure 1**e) <sup>[21]</sup>.



**Figure 1**. Schematic diagram of RecA recombination technique: (**a**) RecBCD binds to the double-stranded DNA terminus and promotes unwinding; (**b**) At the chi site, a single-stranded DNA is produced at the 3' end; (**c**) The RecA protein binds to single-stranded DNA at the 3' end; (**d**) ssDNA-RecA invades intact homologous double-stranded DNA for strand exchange; (**e**) Recombination completed.

Although the RecA-mediated homologous recombination method has shown improved efficiency( $10^{-6}$  to  $10^{-4}$ ) compared to traditional methods for editing the herpesvirus genomes in eukaryotic cells (**Table 1**) <sup>[22]</sup>, it still has significant drawbacks. One major issue is that due to the presence of repeated sequences in the herpes virus genomes, the expression of RecA can lead to instability in the herpes virus's BAC clone, which in turn can cause mutations in the viral genome (**Table 1**) <sup>[1]</sup>. In addition, 500 bp to 3 kb long homologous arms were typically used in RecA-mediated recombination (**Table 1**) <sup>[7][23][24]</sup>, resulting in a cumbersome process for constructing shuttle plasmids required for recombination. These drawbacks limit the application of RecA-mediated recombination.

Editing Techniques	Length of Homologous Arms	Editing Efficiency	Requirement for Editing Site	Stability of BAC
RecA Recombination	500 bp–3 k bp	10 <sup>-6</sup> to 10 <sup>-4</sup>	None	Causes mutations in the BAC
λ-red Recombination	30–50 bp	<1%	None	Maintained the stability of BAC
Base Editing	Not Required	Approaching 100%	PAM Sites & Base Editing Sites; Only Base Editing can be done	Maintained the stability of BAC

**Table 1.** The pros and cons of BAC-based herpesvirus genome editing techniques.

#### 2.2. λ-Red Recombination Technique

Homologous recombination mediated by the  $\lambda$ -red recombination system is currently the most widely used recombination technique for editing herpesvirus BAC clones <sup>[2][25]</sup>. The  $\lambda$ -red recombination system is derived from the *phage*  $\lambda$  and consists of Gam, Exo, and Beta proteins. The Gam protein is an auxiliary protein of Exo protein and Beta protein. The Gam protein can inhibit the binding of Rec BCD to the ends of dsDNA and thus inhibit the function of Rec BCD exonuclease, preventing the degradation of the exogenous double-stranded DNA (**Figure 2**a) <sup>[26][27]</sup>. Exo proteins can bind to the ends of a dsDNA donor, which contains homologous fragments on both sides of the target gene. Concurrently, Exo protein possesses 5' to 3' exonuclease activity, producing a stretch of single-stranded DNA at the 3' end (**Figure 2**b) <sup>[28]</sup>. Beta protein plays a decisive role in the process of  $\lambda$ -red homologous recombination. As a single-stranded DNA binding protein, the beta protein binds to the single-stranded DNA produced by the Exo protein. The binding of Beta protein enhances the annealing of the donor DNA fragment and the homologous sequence at the target site of the replicating herpes virus BAC (**Figure 2**c). Homologous recombination is complete with the replication of DNA (**Figure 2**d) <sup>[29][30]</sup>.



**Figure 2.** Schematic diagram of  $\lambda$ -red recombination technique: (a) Gam protein inhibits the activity of Rec BCD exonuclease; (b) Exo protein creates a stretch of single-stranded DNA at the 3' end; (c) Beta proteins bind here, facilitating annealing interactions between the donor DNA fragment and the homologous sequence of the target site; (d) homologous recombination is complete with the replication of DNA.

Compared with the RecA recombination technique, the  $\lambda$ -red recombination technique avoids the risk of a partial deletion of the herpes virus genome in BAC during the recombination process as only homologous double-strand ends can be used as a substrate (**Table 1**). Additionally, this method typically only requires 30–50 base pairs of homologous arms for recombination (**Table 1**), making it easier to obtain the donor through techniques such as oligonucleotide synthesis or polymerase chain reaction (PCR), eliminating the need for shuttle plasmids as required in RecA recombination <sup>[31][32]</sup>. Importantly, the recombination efficiency mediated by the  $\lambda$ -red recombination technique has been improved to a maximum of 0.68% when the donor is double-stranded DNA (**Table 1**) <sup>[33][34][35]</sup>.

### 2.3. Base Editing Technique

CRISPR/Cas 9 is a powerful genome editing technique <sup>[36]</sup> that has been successfully applied in a wide range of eukaryotic cells, including human cell lines, embryonic stem cells, *mice*, *Arabidopsis*, and *Drosophila* <sup>[37][38][39][40]</sup>. In 2013, Jiang et al. successfully edited *Streptococcus pneumonia*'s genome using CRISPR/Cas-9-only gene editing <sup>[41]</sup>. Since then, it has been successfully employed in a variety of prokaryotic species as well <sup>[42][43]</sup>. The versatility and effectiveness of CRISPR/Cas9 in modifying the genome make it a valuable tool for a wide range of scientific and medical applications.

Due to the lack of the non-homologous end-joining (NHEJ) pathway <sup>[44][45][46][47]</sup> and the low efficiency of their endogenous homologous recombination system, it is difficult to achieve stable genome editing in most bacteria using CRISPR/Cas9 gene editing technology alone <sup>[41][48][49][50]</sup>. Until now, there was still no publication reporting CRISPR/Cas 9-based gene editing technique in the stably preserved herpesvirus BAC gene in *E. coli*. This highlights the need to explore alternative approaches to achieve efficient and reliable gene editing in these organisms.

Recently, scientists have constructed an efficient gene editing method by combing CRISPR/Cas 9 gene editing technique with precise base editing technology. This method consists of an sgRNA and a complex that includes modified Cas9 proteins, cytosine deaminases, and an uracil glycosylase inhibitor (UGI) <sup>[51]</sup>. Unlike wild-type Cas9 proteins, the modified Cas9 is catalytically dead, lacking endonuclease activity. Therefore, it can only facilitate genome targeting via sgRNA but cannot induce a double strand break (DSB) due to the absence of cleavage activity (**Figure 3**a). Cytosine deaminase converts the specified cytosine (C) site to uracil (U) (**Figure 3**b). At this point, uracil glycosylase inhibitors can prevent the excision of intermediate product U, increasing the efficiency of converting C to T on the DNA chain, ultimately achieving single-base precise editing of C to T and G to A (**Figure 3**c).



**Figure 3.** Schematic diagram of base editing method: (a) dCas9 mediates targeting without DSB formation; (b) cytosine deaminase converts C to U; (c) single-base precise editing is complete with the replication of DNA.

Base editing allows for site-directed mutagenesis of multiple prokaryotic genomes <sup>[52]</sup>, including *E. coli* <sup>[22]</sup> and even herpesvirus genome BACs preserved in *E. coli* <sup>[53]</sup>. Zheng et al. <sup>[22][53]</sup> utilized this technology, directly converting cytidine (C) to uridine (U) at specific positions on the US8 and UL34 genes of the pseudorabies virus genome BAC, thus achieving the premature termination of the corresponding genes and approaching 100% editing efficiency (**Table 1**). Due to the modifications that occur in the base-editing window, many studies have demonstrated that base editing is associated with off-target effects <sup>[54][55]</sup>, thereby limiting its practical application. To effectively utilize base editing, optimization of the cytidine deaminase and/or UGI is necessary <sup>[56][57]</sup>. Despite this, the technology has the advantage of directly editing the target site nucleotide without the need for donors. It provides an efficient alternative method for point mutation editing.

Moreover, the novel combined editing technology that has emerged in bacterial genome editing but has not yet been applied to BACs and may also provide insights for future BAC editing. Combining CRISPR/Cas 9 with  $\lambda$ -red recombination could increase the efficiency of recombination in editing the genome of *E. coli*, with reported

knockout efficiency of up to 100% for deletion lengths of up to 3.4 kb, which is higher than in previous reports <sup>[48][58]</sup>. The use of this system could potentially offer a new approach for efficient editing of the BAC of herpesvirus genomes that are stably stored in *E. coli*.

#### References

- 1. Tischer, B.K.; Kaufer, B.B. Viral bacterial artificial chromosomes: Generation, mutagenesis, and removal of mini-F sequences. J. Biomed. Biotechnol. 2012, 2012, 472537.
- Masud, H.M.A.A.; Watanabe, T.; Yoshida, M.; Sato, Y.; Goshima, F.; Kimura, H.; Murata, T. Epstein-Barr Virus BKRF4 Gene Product Is Required for Efficient Progeny Production. J. Virol. 2017, 91, e00975-17.
- 3. Delecluse, H.J.; Hammerschmidt, W. The genetic approach to the Epstein-Barr virus: From basic virology to gene therapy. Mol. Pathol. 2000, 53, 270–279.
- 4. Cohen, J.I.; Wang, F.; Mannick, J.; Kieff, E. Epstein-Barr virus nuclear protein 2 is a key determinant of lymphocyte transformation. Proc. Natl. Acad. Sci. USA 1989, 86, 9558–9562.
- 5. Mannick, J.B.; Cohen, J.I.; Birkenbach, M.; Marchini, A.; Kieff, E. The Epstein-Barr virus nuclear protein encoded by the leader of the EBNA RNAs is important in B-lymphocyte transformation. J. Virol. 1991, 65, 6826–6837.
- Smiley, J.R. Construction in vitro and rescue of a thymidine kinase-deficient deletion mutation of herpes simplex virus. Nature 1980, 285, 333–335.
- 7. Post, L.E.; Roizman, B. A generalized technique for deletion of specific genes in large genomes: Alpha gene 22 of herpes simplex virus 1 is not essential for growth. Cell 1981, 25, 227–232.
- Shizuya, H.; Birren, B.; Kim, U.J.; Mancino, V.; Slepak, T.; Tachiiri, Y.; Simon, M. Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in Escherichia coli using an Ffactor-based vector. Proc. Natl. Acad. Sci. USA 1992, 89, 8794–8797.
- Tsai, M.-H.; Raykova, A.; Klinke, O.; Bernhardt, K.; Gärtner, K.; Leung, C.S.; Geletneky, K.; Sertel, S.; Münz, C.; Feederle, R.; et al. Spontaneous lytic replication and epitheliotropism define an Epstein-Barr virus strain found in carcinomas. Cell Rep. 2013, 5, 458–470.
- Messerle, M.; Crnkovic, I.; Hammerschmidt, W.; Ziegler, H.; Koszinowski, U.H. Cloning and mutagenesis of a herpesvirus genome as an infectious bacterial artificial chromosome. Proc. Natl. Acad. Sci. USA 1997, 94, 14759–14763.
- Borst, E.M.; Hahn, G.; Koszinowski, U.H.; Messerle, M. Cloning of the human cytomegalovirus (HCMV) genome as an infectious bacterial artificial chromosome in Escherichia coli: A new approach for construction of HCMV mutants. J. Virol. 1999, 73, 8320–8329.

- 12. Hosoda, F.; Nishimura, S.; Uchida, H.; Ohki, M. An F factor based cloning system for large DNA fragments. Nucleic. Acids. Res. 1990, 18, 3863–3869.
- Delecluse, H.J.; Hilsendegen, T.; Pich, D.; Zeidler, R.; Hammerschmidt, W. Propagation and recovery of intact, infectious Epstein-Barr virus from prokaryotic to human cells. Proc. Natl. Acad. Sci. USA 1998, 95, 8245–8250.
- Kanda, T.; Yajima, M.; Ahsan, N.; Tanaka, M.; Takada, K. Production of high-titer Epstein-Barr virus recombinants derived from Akata cells by using a bacterial artificial chromosome system. J. Virol. 2004, 78, 7004–7015.
- Delecluse, H.J.; Kost, M.; Feederle, R.; Wilson, L.; Hammerschmidt, W. Spontaneous activation of the lytic cycle in cells infected with a recombinant Kaposi's sarcoma-associated virus. J. Virol. 2001, 75, 2921–2928.
- Wilkinson, M.; Wigley, D.B. Structural features of Chi recognition in AddAB with implications for RecBCD. Cell Cycle 2014, 13, 2812–2820.
- 17. Krajewski, W.W.; Fu, X.; Wilkinson, M.; Cronin, N.B.; Dillingham, M.S.; Wigley, D.B. Structural basis for translocation by AddAB helicase-nuclease and its arrest at chi sites. Nature 2014, 508, 416–419.
- 18. Cox, M.M. Motoring along with the bacterial RecA protein. Nat. Rev. Mol. Cell. Biol. 2007, 8, 127– 138.
- 19. Court, D.L.; Sawitzke, J.A.; Thomason, L.C. Genetic engineering using homologous recombination. Annu. Rev. Genet. 2002, 36, 361–388.
- 20. Ha, T.; Kozlov, A.G.; Lohman, T.M. Single-molecule views of protein movement on single-stranded DNA. Annu. Rev. Biophys. 2012, 41, 295–319.
- 21. Wyman, C.; Ristic, D.; Kanaar, R. Homologous recombination-mediated double-strand break repair. DNA Repair. (Amst.) 2004, 3, 827–833.
- 22. Zheng, K.; Wang, Y.; Li, N.; Jiang, F.-F.; Wu, C.-X.; Liu, F.; Chen, H.-C.; Liu, Z.-F. Highly efficient base editing in bacteria using a Cas9-cytidine deaminase fusion. Commun. Biol. 2018, 1, 32.
- 23. Spaete, R.R.; Mocarski, E.S. Insertion and deletion mutagenesis of the human cytomegalovirus genome. Proc. Natl. Acad. Sci. USA 1987, 84, 7213–7217.
- 24. Mocarski, E.S.; Post, L.E.; Roizman, B. Molecular engineering of the herpes simplex virus genome: Insertion of a second L-S junction into the genome causes additional genome inversions. Cell 1980, 22, 243–255.
- Murata, T.; Isomura, H.; Yamashita, Y.; Toyama, S.; Sato, Y.; Nakayama, S.; Kudoh, A.; Iwahori, S.; Kanda, T.; Tsurumi, T. Efficient production of infectious viruses requires enzymatic activity of Epstein-Barr virus protein kinase. Virology 2009, 389, 75–81.

- Karu, A.E.; Sakaki, Y.; Echols, H.; Linn, S. The gamma protein specified by bacteriophage gamma. Structure and inhibitory activity for the recBC enzyme of Escherichia coli. J. Biol. Chem. 1975, 250, 7377–7387.
- 27. Murphy, K.C. Lambda Gam protein inhibits the helicase and chi-stimulated recombination activities of Escherichia coli RecBCD enzyme. J. Bacteriol. 1991, 173, 5808–5821.
- 28. Little, J.W. An exonuclease induced by bacteriophage lambda. II. Nature of the enzymatic reaction. J. Biol. Chem. 1967, 242, 679–686.
- 29. Karakousis, G.; Ye, N.; Li, Z.; Chiu, S.K.; Reddy, G.; Radding, C.M. The beta protein of phage lambda binds preferentially to an intermediate in DNA renaturation. J. Mol. Biol. 1998, 276, 721– 731.
- 30. Rybalchenko, N.; Golub, E.I.; Bi, B.; Radding, C.M. Strand invasion promoted by recombination protein beta of coliphage lambda. Proc. Natl. Acad. Sci. USA 2004, 101, 17056–17060.
- Yu, D.; Ellis, H.M.; Lee, E.C.; Jenkins, N.A.; Copeland, N.G.; Court, D.L. An efficient recombination system for chromosome engineering in Escherichia coli. Proc. Natl. Acad. Sci. USA 2000, 97, 5978–5983.
- 32. Datsenko, K.A.; Wanner, B.L. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc. Natl. Acad. Sci. USA 2000, 97, 6640–6645.
- 33. Sharan, S.K.; Thomason, L.C.; Kuznetsov, S.G.; Court, D.L. Recombineering: A homologous recombination-based method of genetic engineering. Nat. Protoc. 2009, 4, 206–223.
- 34. Jeong, J.; Cho, N.; Jung, D.; Bang, D. Genome-scale genetic engineering in Escherichia coli. Biotechnol. Adv. 2013, 31, 804–810.
- 35. Pyne, M.E.; Moo-Young, M.; Chung, D.A.; Chou, C.P. Coupling the CRISPR/Cas9 System with Lambda Red Recombineering Enables Simplified Chromosomal Gene Replacement in Escherichia coli. Appl. Environ. Microbiol. 2015, 81, 5103–5114.
- 36. Jinek, M.; Chylinski, K.; Fonfara, I.; Hauer, M.; Doudna, J.A.; Charpentier, E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 2012, 337, 816–821.
- 37. 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. 2013, 31, 230–232.
- Gratz, S.J.; Cummings, A.M.; Nguyen, J.N.; Hamm, D.C.; Donohue, L.K.; Harrison, M.M.; Wildonger, J.; O'Connor-Giles, K.M. Genome engineering of Drosophila with the CRISPR RNAguided Cas9 nuclease. Genetics 2013, 194, 1029–1035.
- 39. Hou, Z.; Zhang, Y.; Propson, N.E.; Howden, S.E.; Chu, L.-F.; Sontheimer, E.J.; Thomson, J.A. Efficient genome engineering in human pluripotent stem cells using Cas9 from Neisseria

meningitidis. Proc. Natl. Acad. Sci. USA 2013, 110, 15644-15649.

- 40. Li, J.-F.; Norville, J.E.; Aach, J.; McCormack, M.; Zhang, D.; Bush, J.; Church, G.M.; Sheen, J. Multiplex and homologous recombination-mediated genome editing in Arabidopsis and Nicotiana benthamiana using guide RNA and Cas9. Nat. Biotechnol. 2013, 31, 688–691.
- 41. Jiang, W.; Bikard, D.; Cox, D.; Zhang, F.; Marraffini, L.A. RNA-guided editing of bacterial genomes using CRISPR-Cas systems. Nat. Biotechnol. 2013, 31, 233–239.
- 42. Altenbuchner, J. Editing of the Bacillus subtilis Genome by the CRISPR-Cas9 System. Appl. Environ. Microbiol. 2016, 82, 5421–5427.
- 43. Huang, H.; Zheng, G.; Jiang, W.; Hu, H.; Lu, Y. One-step high-efficiency CRISPR/Cas9-mediated genome editing in Streptomyces. Acta. Biochim. Biophys. Sin. (Shanghai) 2015, 47, 231–243.
- 44. Shuman, S.; Glickman, M.S. Bacterial DNA repair by non-homologous end joining. Nat. Rev. Microbiol. 2007, 5, 852–861.
- 45. Bowater, R.; Doherty, A.J. Making ends meet: Repairing breaks in bacterial DNA by nonhomologous end-joining. PLoS Genet. 2006, 2, e8.
- 46. Doudna, J.A.; Charpentier, E. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science 2014, 346, 1258096.
- 47. Bikard, D.; Hatoum-Aslan, A.; Mucida, D.; Marraffini, L.A. CRISPR interference can prevent natural transformation and virulence acquisition during in vivo bacterial infection. Cell Host Microbe 2012, 12, 177–186.
- 48. Jiang, Y.; Chen, B.; Duan, C.; Sun, B.; Yang, J.; Yang, S. Multigene editing in the Escherichia coli genome via the CRISPR-Cas9 system. Appl. Environ. Microbiol. 2015, 81, 2506–2514.
- 49. Zerbini, F.; Zanella, I.; Fraccascia, D.; König, E.; Irene, C.; Frattini, L.F.; Tomasi, M.; Fantappiè, L.; Ganfini, L.; Caproni, E.; et al. Large scale validation of an efficient CRISPR/Cas-based multi gene editing protocol in Escherichia coli. Microb. Cell Fact. 2017, 16, 68.
- 50. Bassalo, M.C.; Garst, A.D.; Halweg-Edwards, A.L.; Grau, W.C.; Domaille, D.W.; Mutalik, V.K.; Arkin, A.P.; Gill, R.T. Rapid and Efficient One-Step Metabolic Pathway Integration in E. coli. ACS Synth. Biol. 2016, 5, 561–568.
- 51. Komor, A.C.; Kim, Y.B.; Packer, M.S.; Zuris, J.A.; Liu, D.R. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature 2016, 533, 420–424.
- 52. Gu, T.; Zhao, S.; Pi, Y.; Chen, W.; Chen, C.; Liu, Q.; Li, M.; Han, D.; Ji, Q. Highly efficient base editing in using an engineered CRISPR RNA-guided cytidine deaminase. Chem. Sci. 2018, 9, 3248–3253.

- 53. Zheng, K.; Jiang, F.-F.; Su, L.; Wang, X.; Chen, Y.-X.; Chen, H.-C.; Liu, Z.-F. Highly Efficient Base Editing in Viral Genome Based on Bacterial Artificial Chromosome Using a Cas9-Cytidine Deaminase Fused Protein. Virol. Sin. 2020, 35, 191–199.
- 54. Jin, S.; Zong, Y.; Gao, Q.; Zhu, Z.; Wang, Y.; Qin, P.; Liang, C.; Wang, D.; Qiu, J.-L.; Zhang, F.; et al. Cytosine, but not adenine, base editors induce genome-wide off-target mutations in rice. Science 2019, 364, 292–295.
- 55. Zuo, E.; Sun, Y.; Wei, W.; Yuan, T.; Ying, W.; Sun, H.; Yuan, L.; Steinmetz, L.M.; Li, Y.; Yang, H. Cytosine base editor generates substantial off-target single-nucleotide variants in mouse embryos. Science 2019, 364, 289–292.
- 56. Zuo, E.; Sun, Y.; Yuan, T.; He, B.; Zhou, C.; Ying, W.; Liu, J.; Wei, W.; Zeng, R.; Li, Y.; et al. A rationally engineered cytosine base editor retains high on-target activity while reducing both DNA and RNA off-target effects. Nat. Methods 2020, 17, 600–604.
- 57. Wrighton, K.H. Cytosine base editors go off-target. Nat. Rev. Genet. 2019, 20, 254–255.
- 58. Shukal, S.; Lim, X.H.; Zhang, C.; Chen, X. Metabolic engineering of Escherichia coli BL21 strain using simplified CRISPR-Cas9 and asymmetric homology arms recombineering. Microb. Cell Fact. 2022, 21, 19.
- 59. Dong, H.; Cui, Y.; Zhang, D. CRISPR/Cas Technologies and Their Applications in Escherichia coli. Front. Bioeng. Biotechnol. 2021, 9, 762676.

Retrieved from https://encyclopedia.pub/entry/history/show/97768