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AI-driven Protein Engineering for DNA Sequence Modification

Luqi Lin[1,*], Zhengrong Cui[2], Sihao Wang[3], Yizhi Chen[4], Yanqi Zong[5]

[1]Software Engineering, SunYat-sen University,Shanghai,China
[2]Software Engineering, Northeastern University, Shanghai, China
[3]Mathematics, Southern Methodist University, Dallas, TX,USA
[4]Information Studies, Trine University, Allen Park, MI, USA
[5]Information Studies, Trine University, PhoenixAZ, USA
Corresponding author:Luqi Lin, luqilinhz@gmail.com

Abstract: ***The integration of artificial intelligence (AI) with gene editing technologies like CRISPR-Cas9 holds immense promise for advancing biomedical research and personalized medicine. This article highlights the crucial role of AI in predicting and minimizing off-target effects, thereby enhancing the precision and efficiency of gene editing. Researchers have developed algorithms like BE-DICT to accurately predict base editing outcomes, showcasing the potential of AI-driven strategies in optimizing gene editing processes. By combining AI with bioengineering, this interdisciplinary approach aims to automate and refine DNA modifications, paving the way for innovative applications in personalized gene therapy and biofabrication. Ultimately, this research endeavors to revolutionize the life sciences field, leading to significant breakthroughs in healthcare and biotechnology.***

Keywords: Gene editing; Artificial intelligence; CRISPR-Cas9; Precision medicine.

1. INTRODUCTION

At present, the cutting-edge research of life science and medicine is inseparable from gene editing, and the emergence of base technology makes gene editing more operable. However, the accuracy of the editing site base and the influence of the editing site context sequence on editing have been puzzling researchers. This is related to whether gene editing can be successful, but also affect the clinical effect of gene therapy. Researchers from the University of Zurich have built BE-DICT, an attention-based deep learning algorithm that can predict base editing results with high accuracy.

Artificial intelligence is playing an important role in enhancing gene editing with CRISPR-Cas9 technology. CRISPR-Cas9 is a powerful gene-editing tool that allows researchers to make precise edits to the genome by adding, removing, or altering parts of the DNA sequence. However, one of the challenges with CRISPR-Cas9 technology is to predict and minimize off-target effects, which are unexpected modifications to DNA sequences that are similar to the intended target site.

Artificial intelligence can help solve this problem by analyzing large amounts of genomic data to predict potential off-target effects and their likelihood, leading researchers to more accurate and efficient gene editing. Machine learning algorithms can be trained on large datasets of genomic sequences and [1-4] CRISPR-Cas9 cleavage maps to predict off-target effects based on similarities between target and off-target sequences and other factors such as chromatin accessibility. In addition, AI can assist in identifying optimal target sites for CRISPR-Cas9 editing by analyzing genomic context, functional annotations, and potential off-target sites. This allows researchers to select target sites with minimal risk of off-target and higher editing efficiency.

This research combines artificial intelligence with bioengineering and aims to make DNA modifications using CRISPR-Cas9 gene editing technology [5-7]. We will use artificial intelligence algorithms to analyze genomic data, identify potential editing sites, and design precise editing strategies. Through this interdisciplinary integration, we expect to automate and optimize the gene editing process and improve editing efficiency and accuracy. Our experiments will explore the application potential of AI in the field of bioengineering and provide new ideas and methods for the development of personalized gene therapy and biofabration in the future. This research will promote the integration of gene editing technology and artificial intelligence, bring major breakthroughs in the field of life sciences, and promote the development of health care and biotechnology.

2. RELATED WORK

2.1 Base Editor (BE)

Base editors (BEs) are chimeric ribonucleoprotein complexes composed of a CRISPR-Cas module targeting DNA and a single-stranded DNA deaminase. They facilitate the conversion of C•G base pairs on genomic DNA to T•A pairs, and vice versa. While BEs hold tremendous potential as genome editing tools for both basic research and gene therapy, their application is hindered by the wide variation in editing efficiency across different genomic loci. Researchers conducted a comprehensive analysis of adenine (A) and cytosine (C) base editors using a library containing 28, 294 lentiviral integration gene sequences. They established BE-DICT [8-9], an attention-based deep learning algorithm capable of accurately predicting base editing outcomes. BE-DICT serves as a versatile tool that can be trained on any novel base editor variant, facilitating the application of base editing in both research and therapeutic contexts.

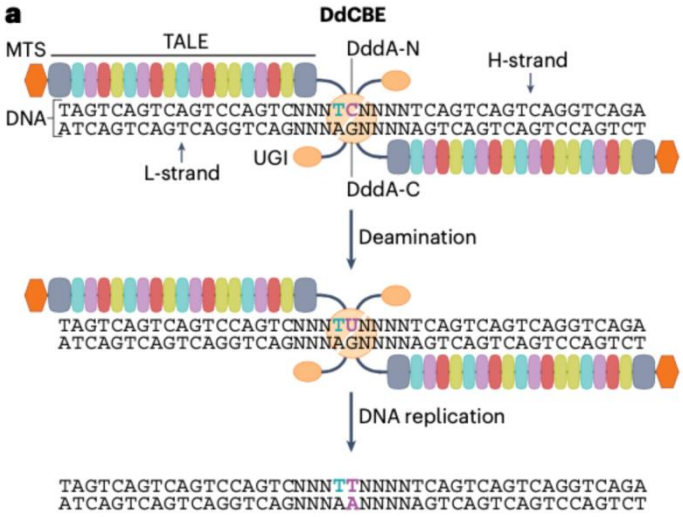

Figure 1: Mitochondrial base editor DdCBE based on double-stranded DNA deaminase DddA

On October 4, 2023, Professors Chen Jia from ShanghaiTech University and Jin-Soo Kim from the National University of Singapore published a comprehensive review article titled "Base editing of organellar DNA with programmable deaminases" in Nature Reviews Molecular Cell Biology. This review provides a detailed overview of the development of cellular DNA base editing tools and their applications in animal mitochondrial DNA and plant plastid DNA [10-15]. It discusses the current limitations of existing tools in terms of efficiency and precision, and explores prospects for their application in disease therapy, agriculture, and environmental contexts.

Furthermore, the integration of artificial intelligence (AI) with genome editing presents significant advantages in advancing this field. AI algorithms, such as deep learning models, can analyze vast amounts of genomic data to predict base editing outcomes with higher accuracy. By harnessing the power of AI, researchers can optimize base editing tools to overcome current limitations and enhance their efficiency and precision.

The synergy between AI and genome editing holds promise for revolutionizing various fields, including personalized medicine, crop improvement, and environmental conservation. By leveraging AI-driven strategies, researchers can accelerate the development of tailored therapies, engineer crops with desired traits, and mitigate environmental challenges more effectively. This convergence of AI and genome editing technologies represents a paradigm shift in biotechnology and offers exciting opportunities for addressing pressing global challenges.

2.2 Gene editing applications

The history of gene therapy has been marked by both triumphs and setbacks since the inception of the Human Genome Project in the 1990s. Despite early optimism, tragedies such as the death of Jesse Gelsinger in 1999 during a gene therapy clinical trial at the University of Pennsylvania cast a shadow over the field, leading to years

of skepticism and caution. However, recent breakthroughs in gene therapy have reignited hope and demonstrated its potential to revolutionize medicine.

One remarkable success story is the advent of CAR-T tumor immunotherapy, a form of gene therapy that has revolutionized the treatment of hematological malignancies. By genetically modifying a patient's own immune cells to recognize and attack cancer cells, CAR-T therapy has shown unprecedented efficacy in treating previously incurable cancers [16].

Similarly, the approval of Zolgensma, a gene therapy for spinal muscular atrophy, has provided a beacon of hope for thousands of children suffering from this devastating disease. This groundbreaking therapy, which targets the underlying genetic cause of the disease, has not only improved patients' quality of life but also demonstrated the commercial viability of gene therapy, with global sales exceeding $ 1.4 billion in 2022.

Despite these successes, many rare and neglected diseases continue to afflict marginalized communities, often due to a lack of financial support and research infrastructure. Sickle cell disease, one of the most well-known genetic disorders worldwide, exemplifies this disparity [17-19]. Caused by a single mutation in the hemoglobin beta chain gene, sickle cell disease results in the production of abnormal hemoglobin that causes red blood cells to deform under low oxygen conditions, leading to vaso-occlusive crises and organ damage.

Here, the promise of DNA gene editing emerges as a potential solution to address the root cause of sickle cell disease. By precisely correcting the genetic mutation responsible for the disease, gene editing technologies offer the possibility of curing sickle cell disease at its source. Moreover, advancements in gene editing tools, such as CRISPR-Cas9, have made targeted gene correction more accessible and efficient, paving the way for personalized therapies tailored to individual patients.

In conclusion, while the journey of gene therapy has been fraught with challenges, recent breakthroughs underscore its transformative potential in treating a wide range of diseases, including those that have long been neglected. By harnessing the power of DNA gene editing, we can pave the way towards a future where genetic disorders like sickle cell disease are no longer a barrier to health and well-being.

2.3 CRISPR-Cas9 gene editing

CRISPR Cas9 gene editing technology is a revolutionary technology that has attracted much attention at present, and its principle and application have widely influenced the development of the life science field. The technique, derived from a natural immune system in prokaryotes, is highly specific and efficient, able to accurately identify and cut specific DNA sequences in the genome [20-23]. In the CRISPR Cas9 system, the Cas9 protein is responsible for cutting DNA, while crRNA and tracrRNA combine to form a gRNA that directs the Cas9 protein to find the target DNA and make the cut. In order to facilitate experimental design and improve the stability of gRNA, the scientists combined crRNA and tracrRNA into a single sgRNA, which greatly simplifies the application process of CRISPR Cas9 technology.

The wide application of CRISPR Cas9 technology is due to its efficiency and flexibility. By designing different gRNA sequences, it is possible to accurately locate specific sites in the genome to achieve gene knockout, editing or repair. This allows scientists to study gene function and disease mechanisms in greater depth, and provides powerful tools for fields such as gene therapy and agricultural breeding.

In addition to its applications in basic science research [24], CRISPR Cas9 technology also shows great potential in the medical field. For example, it is used to treat some genetic diseases, such as cystic fibrosis and hereditary deafness. By repairing the pathological genes in the somatic cells of patients, effective treatment programs can be provided for patients. In addition, CRISPR Cas9 technology is being used in cancer research and drug development to help scientists better understand tumorigenesis mechanisms and develop new treatments.

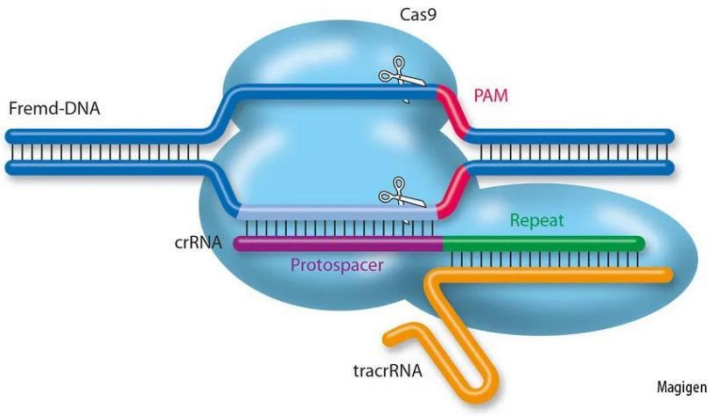

Figure 2: How CRISPR Cas9 gene editing works

However, despite the huge potential of CRISPR Cas9 technology, there are some challenges and limitations to its application [25]. For example, there are still questions about the accuracy and efficiency of gRNA design, as well as safety issues such as non-specific cutting and cytotoxicity that may result. In addition, applications in areas such as gene editing and gene drives in human embryos have also sparked ethical and legal controversies.

Overall, CRISPR Cas9 gene editing technology is a revolutionary biotechnology that brings new hope and opportunities in fields such as genome editing and precision medicine. As the technology continues to develop and improve, it is believed that CRISPR Cas9 technology will play an increasingly important role in the future, making greater contributions to human health and the progress of biological science.

3. METHODOLOGY

3.1 How CRISPR-Cas9 gene editor works

The CRISPR-Cas9 gene editing technology uses an immune system naturally found in bacteria and archaea to fend off viral invasions. The system consists of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and Cas proteins. The CRISPR sequence is a special sequence that preserves fragments of viral DNA inside bacterial and archaea cells, while the Cas protein is the enzyme responsible for recognizing and cutting foreign DNA. The working process can be divided into three main steps: the first step is to form a complex of Cas9 protein and RNA on CRISPR sequence through RNA guidance [26-29]; The second step is for the complex to recognize and bind to the target DNA sequence, where RNA acts as a guide, instructing Cas9 to bind to the target DNA; The third step is for the Cas9 protein to cut the target DNA to enable gene editing.

The Cas9 protein in the CRISPR-Cas9 system is an RNA-guided DNA endonuclidenase that is able to bind to two types of RNA: RNA transcribed by CRISPR sequences (called crRNA) and a helper RNA (called tracrRNA). These two Rnas form a complex, called a single guide RNA (sgRNA), whose function is to guide the Cas9 protein to bind to the target DNA sequence. Once the sgRNA binds to the target DNA, Cas9 forms a double-stranded incision at a specific location in the target DNA, resulting in a double-stranded DNA break. Repair mechanisms in the cell attempt to repair these breaks, often triggering the insertion or deletion of DNA bases to enable editing of the genome.

The key to CRISPR-Cas9 gene editing technology is its highly customizable nature. By synthesizing different sgRNA sequences, the Cas9 protein can be directed to specific locations in the genome, enabling accurate editing of the gene. This highly accurate editing capability makes CRISPR-Cas9 technology promising for a wide range of applications in genome editing, disease modeling, and biointelligent engineering.

3.2 The advantages of CRISPR-Cas9 in gene editing

The specific action mechanism of CRISPR-Cas9 in gene editors can be divided into the following three stages [2]:

Access to highly variable spacer regions of CRISPR: When invaded by foreign phages or plasmids, CRISPR-containing bacteria and archaea acquire foreign DNA fragments inserted into spacer regions;

Expression of CRIPSR loci: The spacer homologous foreign nucleic acid re-enters the bacteria and activates CRISPR array transcription to produce pre-crRNA [30]. At the same time, tracrRNA, which is complementary to the pre-crRNA sequence, is also transcribed. After transcription, tracrRNA first binds to Cas9 protein. Complementary base pairs between pre-crRNA and tracrRNA form double-stranded RNA, which then binds to Cas9 to form a complex.

After the double-stranded RNA binds to Cas9, RNase III builds pre-crRNA in the primary process, and the secondary processing of Cas9 can reduce the redundant repeat sequence and interval sequence. After these two processes, crRNA matures and gains the ability to target DNA strands.

Activation of the CRISPR/Cas system (targeted interference): If reinfected with homologous DNA, the bacteria will initiate transcription of the CRISPR region. After a series of processing and maturation processes to produce sgRNA, sgRNA guides Cas9 to cut DNA strands that destroy homologous septal regions leading to DSB, which cells repair through NHEJ or HR.

3.3 Classification of CRISPR gene editing functions

DNA strand cutting tool. CRISPR/Cas9 was originally studied because of its powerful double-stranded DNA cutting function. sgRNA directs Cas9 to a designated site where DSBs form flush ends in the presence of HNH and RuvC nuclease domains. It was originally widely studied for its powerful double-stranded DNA cutting function. Cas9 is guided to a specific site by sgRNA so that it forms a double-strand break at that location, forming a flush end under conditions of HNH and RuvC nuclease domains. Subsequently, DNA repair mechanisms are activated, primarily through non-homologous end joining (NHEJ) and homologous recombination repair (HDR). NHEJ repair is imprecise and often results in base mutations, resulting in unintended mutations. HDR repair is a complex and precise process that correctly repairs broken DNA strands. A fully repaired strand of DNA is indistinguishable from the target DNA, so it may be cut again by Cas9 until it is no longer recognized by the sgRNA. Fortunately, the chance of HDR occurring in mature cells is low.

Although Cas9 is effective at cutting double-stranded DNA [31], in practice, mismatching sgRNA with DNA can lead to off-target effects. To reduce this off-target effect, and to improve the efficiency of gene knockout, Cas9 nickase (Cas9n) was introduced, a variant of Cas9 with a mutation in its RuvC nuclease domain (D10A) that resulted only in a break in the DNA strand that was complementary to crRNA. DNA single strand breaks are usually repaired by the high-fidelity base excision repair (BER) pathway. Therefore, designing two adjacent sgRNA/Cas9n complexes to shear a single site can effectively prevent Cas9-mediated damage to non-target DNA and greatly enhance Cas9 specificity. Properly spaced Cas9n complexes help improve the efficiency of gene editing, which demonstrates the advantages of DNA gene modification while preserving the original core function.

3.4 Gene expression regulation and epigenetics

CRISPR/Cas9's ability to cut double-stranded DNA depends on two nuclease domains, mutations of which cause loss of enzyme-mediated cutting activity to dCas9. These mutants are still able to bind to specific sites on the DNA chain under the guidance of RNA and affect gene transcription, but there are no serious off-target effects. Since Cas9 has been shown in other studies to load a large number of proteins to a specific location in the genome and perform its function, designing a transcription factor binding dCas9 to regulate the expression of target genes is a potential research direction to realize the application of [32] CRISPR/dCas9 systems. Many diseases are often accompanied by high expression of inflammatory factors or harmful genes during development, and inhibiting the expression of this activated or restored protective gene is important to target certain chronic diseases. In addition, unlike CRISPR/Cas9 gene editing, CRISPR activation (CRISPRa) and CRISPR interference (CRISPRi) are reversible because the genome is not modified, This greatly reduces the unknown problems caused by off-target effects. Because the base sequence of DNA is not directly changed, the efficiency of gene editing limits the application of CRISPR/dCas9.

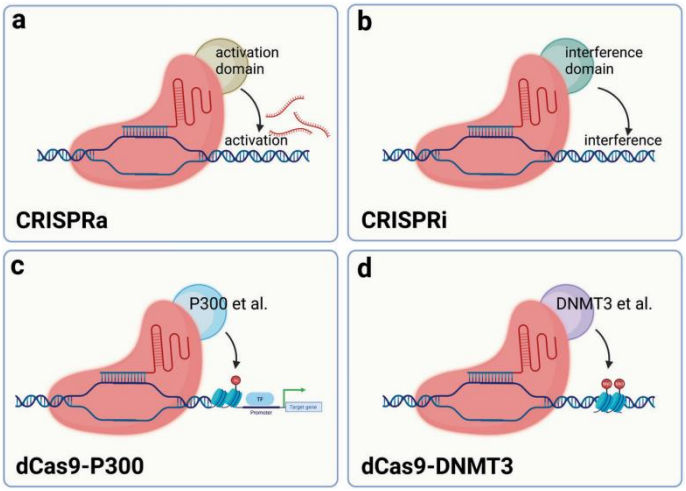

Figure 3: Gene expression regulation and epigenetic modification tool set

Gene regulation in eukaryotes is a complex process in which most genes are controlled by multiple interacting regulatory elements. Epigenetic modifications also affect gene expression. Gilbert et al. fused dCas9 with multiple inhibitory chromatin modification domains and screened KRAB(ruppel-associated box), a repressor domain that significantly inhibited gene transcription. CRISPR/ dCas9 binding to the activated domain also promotes gene expression; Both VP64, which consists of four copies of the transcriptional activator VP16, and p65, which activates the domain, enhance transcription. Multiple activation or inhibition domains have been developed to regulate gene expression. CRISPR/ dCas9 is a general-purpose transcriptional regulatory platform that can load active or inhibitory domains to regulate gene expression. In addition, epigenetic modifications may also be regulated by DCas9-supported epigenetic modification enzymes such as DNA methyltransferase DNMT3A and acetyltransferase P300. CRISPR/dCas9 has a much lower risk of off-target effects than Cas9 and the effects are relatively efficient and mild, but the mechanisms regulating gene expression are very complex. Therefore, designing an sgRNA that targets one site can result in the expression of multiple genes, and its risks must be further explored by conducting more in-depth studies (Figure 3).

At present, the cutting-edge research of life science and medicine is inseparable from gene editing, and the emergence of basic technologies makes gene editing more operational [33]. However, the accuracy of gene editing sites and the effect of editing site context sequences on editing have been puzzling researchers. This is related to the success of gene editing and also affects the clinical effect of gene therapy. Researchers at the University of Zurich built BE-DICT, a deep learning algorithm based on attention mechanisms that can predict base editing outcomes with high accuracy.

4. CONCLUSION

Artificial intelligence plays an important role in enhancing gene editing with [34-35] CRISPR-Cas9 technology. CRISPR-Cas9 is a powerful gene-editing tool that enables researchers to precisely edit the genome by adding, removing, or altering parts of the DNA sequence. However, one of the challenges with CRISPR-Cas9 technology is to predict and minimize off-target effects, which are accidental modifications to DNA sequences that are similar to the target site.

Ai can predict potential off-target effects and how likely they are to occur by analyzing vast amounts of genomic data, allowing researchers to make gene editing more accurate and efficient. Machine learning algorithms can be trained on datasets of large genome sequences and CRISPR-Cas9 [36] cut maps to predict off-target effects based on similarities between target and off-target sequences, as well as other factors such as chromatin accessibility. In addition, AI can help identify the best targets for CRISPR-Cas9 editing by analyzing genomic context, functional annotations, and potential off-target sites. This allows researchers to select targets with minimal risk of off-target and higher editing efficiency.

The research combines artificial intelligence with bioengineering and aims to make DNA modifications using CRISPR-Cas9 gene-editing technology [37]. We will use AI algorithms to analyze genomic data, identify potential editing sites, and design precise editing strategies. Through this interdisciplinary integration, we expect to

automate and optimize the gene editing process, improving editing efficiency and accuracy. Our experiments will explore the application potential of artificial intelligence in the field of bioengineering and provide new ideas and methods for the development of personalized gene therapy and biofabration. This research will promote the integration of gene editing technology and artificial intelligence, making major breakthroughs in the field of life sciences and advancing the development of healthcare and biotechnology.

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