iJRASET
International Journal For Research in Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 11 **Issue:** VII **Month of publication:** July 2023

DOI: https://doi.org/10.22214/ijraset.2023.55035

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 11 Issue VII Jul 2023- Available at www.ijraset.com

CRISPR-Cas9 Gene Editing: Current Progress and Future Applications

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Abstract: ***Because of the groundbreaking gene-editing software clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9 (CRISPR-Cas9), the field of molecular biology has been altered in a revolutionising way. It holds unparalleled potential for applications in a wide range of factors, including but not limited to biotechnology, agriculture, and medicine, since it allows for exact modifications to live creatures' DNA. This paper looks at the current state of CRISPR-Cas9 gene editing, its mechanics, recent advances, and ethical considerations. It also analyses the technology's potential future applications, emphasising its impact on human health, agriculture, and environmental protection.***

I. INTRODUCTION

In today's generation, gene editing has witnessed remarkable progress with the advancement of CRISPR-Cas9 technology. CRISPR-Cas9 is a gene editing technology that allows biologists, medical researchers and geneticists to edit parts of the genome and revise fallacies in the same. This revolutionising technology has created quite the roar in the scientific world by being the most multiverse, flexible and meticulous method of genetic modification. This section provides an overview of the fundamental principles of CRISPR-Cas9 and its historical development, outlining its significance in the context of genetic engineering. Investigating the historical development, the first experimental information pertaining to the technologies mechanism is dated back to 2007 in the studies of Rodolphe Barrangou and Philippe Horvath, two French culinary scientists researching yoghurt cultures of bacteria *Streptococcus thermophilus* for Danisco (Danish bio-based food production company). CRISPR-Cas9 has extreme significance in the world of genetic engineering, as it aids in tailoring genes to rectify mutations or examine gene function. CRISPR-Cas9 requires two quintessential elements to introduce a mutation: a guide RNA (gRNA), an enzyme by the name of Cas9 - essentially a endonuclease which causes a double stranded DNA break- that can cut DNA at specific locations of the genome to permit the addition or subtraction of DNA.

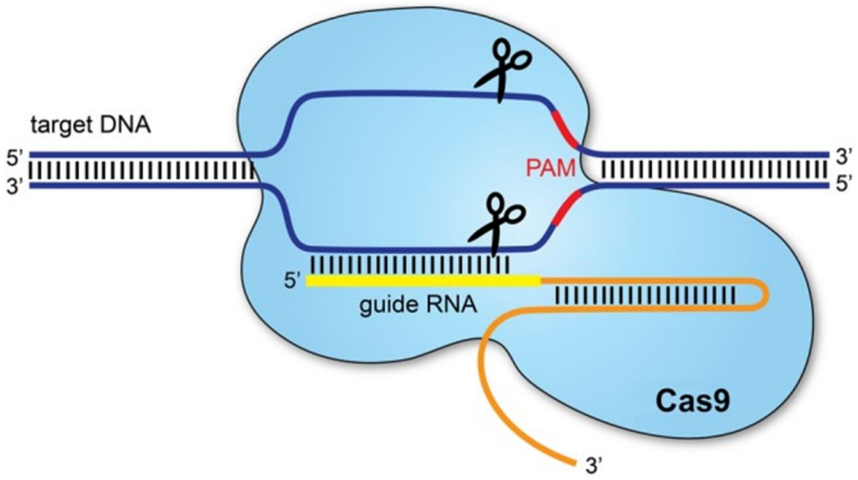


Fig.1 The CRISPR-Cas9 System

Cas9 associates with the gRNA to form a complex to allow accurate selection of a desired site in the DNA.The process initiates as the complex binds to the original target DNA. This allows for the unwinding of the DNA helix allowing the guide RNA to pair with the target-specific sequence in the DNA. The Cas9 enzyme then proceeds to cut both strands of DNA, and as part of the mending process the cut is repaired through a mutation.

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II. CURRENT APPLICATIONS

One of the most notable advancements in the scientific world using CRISPR-Cas9 is gene therapy for various diseases. CRISPR therapy has been successful with treating patients suffering with sickle cell anaemia. This disease manifests itself through mutations caused in the gene that produces beta-globin. As the primary source of the difficulty is due to a mutation in the gene, CRISPR-mediated therapy is an ideal solution to treat the disease.

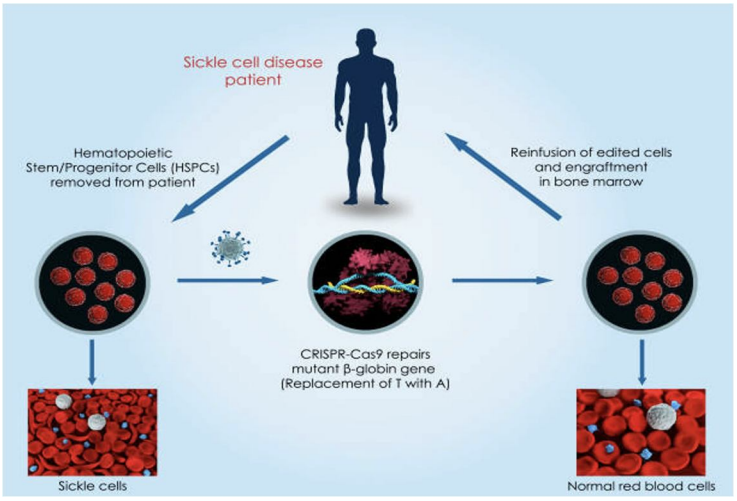


Fig. 2 Approaches to CRISPR Sickle Cell Disease Gene Therapy

Treating the mutation requires an ex vivo procedure, through which hematopoietic stem cells are extricated, corrected and restored back to the patient. Apart from sickle cell anaemia, CRISPR-Cas9 technology has shown promise for oncology patients as well. The field of cancer immunotherapy has shown positive results in the treatment of tumours, and researchers are constantly exploring ways to enhance its effectiveness. By utilising the CRISPR-Cas9 system, it is possible to increase the pool of available CAR-T cells for patients with cancer, thus improving treatment options [1]. In addition, interrupting genes that encode T-cell signalling molecules or inhibitory receptors using CRISPR/Cas9 can enhance CAR-T cell function in cancer immunotherapy [1]. The current applications of CRISPR/Cas9 in cancer immunotherapy are being studied and utilised, with ongoing clinical trials focusing on various types of cancers such as brain cancer, renal cell carcinoma, colorectal cancer, hepatocellular carcinoma, urinary bladder cancer, and more [1]. The role of CRISPR/Cas9 in enhancing cancer immunotherapy is still being explored, with researchers investigating the potential benefits and future prospects of this technology in improving treatment outcomes for cancer patients [1]. Factors such as the spatial and temporal control of the CRISPR system, DNA end structure and sequence features, DNA repair mechanisms, and other variables can influence the outcome and efficiency of gene editing using CRISPR/Cas9 [2]. Furthermore, clinical trials that combine cancer immunotherapy with the CRISPR/Cas9 system are currently underway, highlighting the potential of this approach in revolutionising cancer treatment [2]. Overall, CRISPR/Cas9 has emerged as a powerful tool with potential applications in enhancing various aspects of cancer immunotherapy, ranging from improving antibody performance to modifying the tumour microenvironment and immune responses [2].

III. FUTURE DIRECTIONS

The future of CRISPR-Cas9 holds immense promise for future researchers and medical practitioners, the new software could be heading in multiple different directions in the future, one including base editing and prime editing. Base editing represents a powerful tool within the CRISPR-Cas9 genome editing system, enabling precise and targeted changes to individual nucleotides within the DNA sequence. Unlike traditional CRISPR-Cas9 techniques that introduce double-stranded breaks in the DNA, base editing offers a more controlled approach by directly modifying specific bases without altering the DNA backbone. By utilising a modified version of Cas9, known as a base editor, scientists can selectively change one nucleotide to another or convert cytosine to uracil. The base editor consists of three key components: a catalytically impaired Cas9 protein, an engineered enzyme capable of deaminating cytosine, and an RNA molecule that guides the complex to its target site. This combination allows researchers to achieve precise base modifications by leveraging the cell's natural repair mechanisms.

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Prime editing represents a significant advancement in the field of gene editing, expanding on the capabilities of CRISPR-Cas9 technology. Developed by Dr. David Liu and his team at the Broad Institute, prime editing offers precise modifications to the DNA sequence without requiring a double-stranded DNA break. The technique utilises an engineered Cas9 enzyme fused with a reverse transcriptase, along with an RNA guide that directs the enzyme to the target site. The reverse transcriptase component then generates a new DNA strand by copying an RNA template embedded within the guide RNA molecule. This newly synthesised DNA strand replaces the original sequence at the target site, resulting in precise base changes or insertions. Prime editing holds immense potential for correcting genetic mutations associated with various diseases, including those previously considered challenging to address using traditional CRISPR-Cas9 methods.While the benefits of CRISPR are boundless, the ethical challenges that pull this technology back aren’t any less.

The emergence of CRISPR-Cas9 as a powerful gene-editing tool has raised profound bioethical concerns. One major issue revolves around the potential for germline editing, which allows modifications to be passed down to future generations. This raises questions about the ethics of altering human traits and potentially creating "designer babies." Moreover, unintended off-target effects and the potential for genetic discrimination pose additional ethical challenges. The question of equitable access to this technology is also crucial, as it may exacerbate existing social inequalities. Furthermore, there is a pressing need for regulatory frameworks that can balance scientific progress with ethical considerations, ensuring responsible use and preventing misuse or abuse of this transformative technology.

IV. CONCLUSION

Keeping in mind the multiple aspects both positive and negative of the CRISPR-Cas9 technology, it proves to be a promising gene editing technology in the future years. Showing benefits in various diseases including sickle cell anaemia and cancer. While the ethical argument of whether “designer babies” are a morally just way of using the technology persists to remain in debate, this software does show promising benefits in the future.

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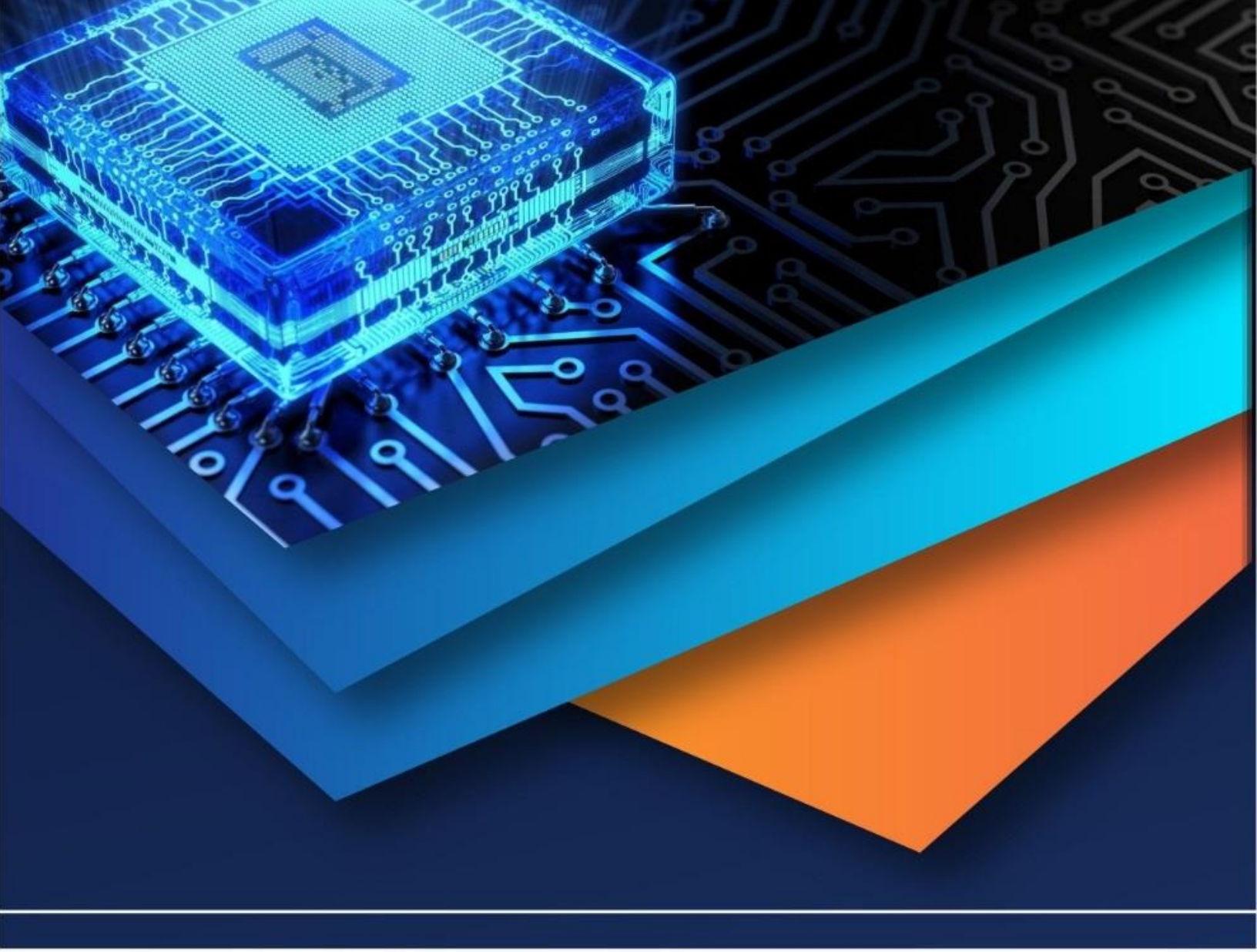
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