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Survey on the CRISPR Technology

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Abstract -Genetic Engineering is one of the important concepts in healthcare services as it is used to manipulate genes implying It is used to eliminate or alter harmful genes from the organisms. CRISPR is one such Genetic Engineering technique which edits and cuts genome in living cells. CRISPR is helpful in curing many diseases, including cancer, but it has many disadvantages too, as sometimes the DNA which has been edited doesn't behave correctly or sometimes it cuts the wrong DNA which results in worsen the diseases. Here, we present a brief history of CRISPR-Cas9, how does it work? And how it can be more accurate using Machine Learning algorithms which will help in finding out the off-target effects. Furthermore, we Survey current applications of CRISPR and discuss its usefulness in curing fatal diseases and crop improvement.

Key Words:CRISPR, Genetic Engineering, Gene-editing, gene, DNA

1. INTRODUCTION

DNA stands for Deoxyribonucleic acid, it contains all the genetic information, also known as genetic code, of an organism. Gene is the building block of any heredity which gets transferred from parents to their children or offspring which defines the feature of each child or off-spring. Gene is made up of DNA whereas Genome is a whole set of DNA i.e., more than 3 billion DNA base pairs. Each genome contains all the features/properties of an organism that needs to maintain and build that organism and it is present in each cell's nucleus [20].

CRISPR is gene editing, gene modification, a technology which means being able to edit or cut any specific gene and improve the quality of genome. Genome. CRISPR stands for "Clustered Regularly Interspaced Short Palindromic Repeats" which is gotten from a bacterial resistance system that forms the reason for CRISPR-Cas9 genome modification innovation[5][3][22].

CRISPR has two primary segments a DNA-cutting protein called Cas9 framework and an RNA particle known as the Guide RNA(gRNA). They, Both together, they can determine and cut explicit areas of DNA. Cas9 is a nuclease, a type of enzyme that can cut DNA, at first it locates and binds a common sequence in DNA called PAM, Protospacer adjacent motif is a short DNA sequence usually 2-6 base pairs in length, then unbinds the double helix DNA and matches to its target RNA. If the match is complete, the cas9 utilizes its two tiny molecular scissors to cut the DNA. Guide RNA or gRNA presents target sequence specificity to the CRISPR-Cas9 technology. These gRNAs are non-coding short RNA sequence that binds to the correlative objective DNA sequence. Guide RNA first ties to the Cas9 chemical and the gRNA grouping guides the complex by means of matching to a particular area on the DNA, where Cas9 plays out its endonuclease action by cutting the objective DNA strand[1][2][19][22].

The objective of this review is to discuss the primary features of the CRISPR/Cas9 genome editing technique and its application in different fields such as treating diseases in human beings and improving crop genome in plants.

2. BACKGROUND

CRISPRs were first discovered in 1987 when Japanese researchers examining E. coli bacteria originally went over some uncommon repeating sequences in the bacteria's DNA. "The biological significance of these sequences," they stated, "is Unknown"[14]. Over time, different researchers discovered comparative bunches in the DNA of other bacteria (and archaea). They gave these successions a name: Clustered Regularly Interspaced Short Palindromic Repeats — or CRISPR[18][22].

CRISPRs serve as part of the bacterial immune system, defending against invading viruses. Bacteria are under constant assault from viruses, in order that they produce enzymes to repel viral infections. Whenever a bacterium's enzymes manage to exterminate an invading virus, other little enzymes will come along, scoop the remains of the virus's ordering and cut it into tiny bits. The enzymes then store those fragments in CRISPR spaces within the bacterium's own genome[8][10][19].

For quite a long while, the role of the short repeat sequences remained unchanged, until in 2005 a few studies portrayed similarities of these successions to phage DNA, raising the theory that these sequences are a part of an adaptive immune system in bacteria. These examinations were later reached out to experimentally demonstrate that CRISPR and its CAS, CRISPR-associated proteins, are connected to the adaptive immunity targeting foreign viral DNA [16]. Mechanistically, two particular RNAs – the CRISPR focusing on (crRNA) and the trans-activating RNA (tracrRNA) – enact and manage Cas proteins to tie viral DNA arrangements which are in this way severed. CRISPR/Cas9 gene-editing systems were discovered in animal and plant biology with its utility in genome editing in 2012 in mammalian cells. CRISPR genome altering is straightforward unlike ZFNs and TALENs, it

includes structuring a guide RNA (gRNA) of around 20 nucleotides corresponding to the DNA stretch inside the target gene[2][11].

The race between bacteria and phages has driven the advancement of CRISPR–Cas systems, bringing about six essential kinds of CRISPR–Cas systems. Type I, II, III, IV, V, and VI where Types I, III, and IV are characterized by multi-subunit effector complexes, while types II, V, and VI are regarded as a single-subunit effector. The type II CRISPR/Cas9 system from spCas9, stands for Streptococcus pyogenes, is a rising genome altering instrument with wide applications because of its proficiency, simple to take care of, and straightforwardness [21].

3. MACHINE LEARNING MEETS CRISPR

The basic idea behind CRISPR was to edit or cut specific DNA sequences to improve the genome quality or fix the harmful mutation. But even in system like CRISPR which prioritizes exactness, sometimes it can lead to mistakes. The James Zou, PhD, assistant professor of biomedical data science, and collaborators have made an algorithm that predicts what sort of mistakes are probably going to happen during CRISPR altering. The paper "Large dataset enables prediction of repair after CRISPR–Cas9 editing in primary T cells", specifying the work shows up in Nature Biotechnology. This work is a joint effort with scientists at the Chan Zuckerberg Biohub and the University of California, San Francisco and Zoe was the senior author[4][7].

When we edit or cut any gene using CRISPR then a strand of molecules also known as guide RNA(gRNA) drives the DNA-cutting protein Cas9 to the segment of DNA which is targeted for editing. RNA locates and binds a common sequence in DNA called PAM, then unbinds the double helix DNA and matches to its target RNA. If the match is complete, the Cas9 utilizes its two tiny molecular scissors to cut the DNA so that new DNA can be inserted or deleted. But this process is error-prone and leads to harmful mutations of genes, In some cases, nucleotides (the building block of DNA) are lost, and in other cases, they are mysteriously attached. This can be dangerous to a specific cell and even the organism overall[1][15].

That's where the James Zou machine learning algorithm, called CRISPR Repair Outcome, or SPROUT, comes into the picture to determine what will be the unexpected changes and whether they will be harmful?. "There could be a lot of arbitrariness in what occurs during these CRISPR alters, and that arbitrariness can conceivably make startling results," said Zou. "So our work is persuaded by whether we can measure those chances all the more exactly"[7][15][18].

4. APPLICATIONS OF CRISPR

Crop Improvement - According to the Food and Agriculture Organization of the United Nations, In 2018, more than 700 million of the world's population were exposed to severe levels of food insecurity which means a reduction in the food quality consumed by them and possibly they have experienced hunger. Furthermore, 1.3 Billion people experienced irregular access to nutritious and sufficient food. CRISPR technology is one of the revolutionary technology that can improve crop improvement and increase production to some extent. It has been already implemented in nearly 20 crops which includes staple food, a major cereal crop, fiber crop, seed oil crop, etc. [5][6][17].

Curing Diseases - CRISPR is tackling fatal diseases like Cancer and HIV. One of the biggest achievement so far has been the elimination of HIV from the human cell. A team of researchers, at Lewis Katz School of Medicine at Temple University (LKSOM), removed HIV-1 DNA from T cell genomes in human lab cultures. In fact later on, when these cells were made in contact with the virus they did not get re-infected implying virus is prone to re-infect victims[9][23].

Many researchers are working on the removal of Malaria from the mosquitoes so it doesn't affect humans. Using CRISPR, a biologist can cut the genes which lead to the spread of malaria within the mosquito population. The scientific journal Nature Biotechnology declared the use of a new method called "gene drive". It can also be used to treat genetic blindness as many genetic blindness happens due to a particular mutation, that is not a big deal for CRISPR-Cas9 to target a specific region of cell, or a gene, and edit it [23][24].

5. CONCLUSION

The Story of the CRISPR technique has gained the researcher's attention pretty quickly and became one of the powerful technologies in Genetic Engineering. This article has an overview of CRISPR technology as to how it works, when it is first discovered, why is it called CRISPR, and different applications of it in different domains, which will help the students to know about CRISPR in a short span of time. The use of Machine learning in CRISPR is unavoidable, It is important for researchers or scientists to know all the off-target risks, which can affect the crop or human, before manipulation of the gene.

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