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CRISPR Technology: Advantages, Limitations and Future Direction

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Abstract

The evolutionary discovery of CRIPRS technology has paved the way for researchers in various fields' genetics, medicine, pharmacy and computer science. Its potential application is limitless spanning from therapy of disease such as cancer and immunotherapy with gene silencing, gene knock down, gene KO, to the food production of genetically modified foods, the potential possibility of genetically designing baby using CRSIPR technology with enhanced trait and potential of eradicating Malaria and HIV-AIDS. The review thereby focuses on Off-targets limitation of the technology which is explained as one of the major constraint for application in clinical procedure with its hope of eradication through machine learning (ML). Further application of crisper technology was also discussed.

Keywords: CRISPR; Designer Baby; Machine Learning; Off Targets; Gene Knock out

Introduction

The word "smart" has been the recent trend in almost all technological industry. Nowadays, we have smart phones, smart TV, smart houses and others. Is it too soon to start having smart babies? The potentiality of having smart babies or designer babies as some researchers would describe it is due to the discovery of CRISPR-Cas9 gene editing technology. Clustered Regularly Interspaced Short Palindromic Repasts (CRISPR) is home to a wide of application and potential with the promise of altering genes to produce desired trait and treatment of diseases (such as cancer, genetic disorder). The mechanism aging has been showed to be hindered by CRISPR technology. Other application of CRISPR extends to food and agriculture in the control of pest, modification of plants in terms of GM (Genetically Modified) foods [1].

History of CRISPR technology

CRISPR Technology came into being as a result of a basic research project that was aimed at discovering the mechanism of how bacterial cell fight viral infection such as Bacteriophages. Before the Invention and revolution of CRISPR technology, so many scientists have contributed widely on gene editing techniques [2] (Doudna Et al 2017). CRISPR which stand for Clustered Regularly Interspaced Short Palindromic Repeat are sequences of DNA which were initially discovered in the *E. coli* genome in 1987 by Japanese scientist Yoshizumi Ishino and his colleagues during an experiment and analysis of gene responsible for isozyme conversion of alkaline, but the function of the repeated sequence was not known to them. These repeated sequences are named short regularly spaced repeats (SRSS) in 2000. Same year some group of scientists discover another type of gene editing tool known as zinc finger nucleases. Not only in Bacteria, CRISPR is also present in archaea, this discovery is made by Mojica and his colleagues during an experiment using *Haloferax mediterranei* an archaea to adapt in a high salt environment [3]. They also discovered the similarities between CRISPR's spacer regions and sequences of bacteriophages, archaeal viruses, and plasmids and this discovery led to the understanding of the function of CRISPR in relation to immune system [4]. After the validation of the gene, the SRSS was renamed CRISPR in the year 2002 by Jansen and his colleagues, couple with renaming SRSS, scientist also discovered a gene call Cas which translate a protein that form a complex with a guide RNA and has a scissors edge that cut DNA into fragment [5]. It wasn't untill not until between the year 2005 and 2007 Mojica and other scientist understand that prokaryotes used CRISPR as part of an adaptive immune system and the discovery of Cas9 and PAM by Bolotin. The process where bacterial CRISPR transcribed a guide RNA and form CRISPR-Cas9 Complex which not only store a record of invading bacteriophages and other viral DNA but also to destroy the viruses upon second attack [6]. A group of scientists working at a yogurt and cheese industry encountered a decrease in yield as the bacteria *S. thermophilus* used in the process is attacked by a virus; Phillipe Horvath observed some of the bacteria are immune to virus and stored a Viral DNA into their CRISPR [7]. A group of scientist from University of Laval showed that CRISPR along with Cas9 created double strand breaks in target DNA at a precise position with three nucleotide upstream of the PAM. CRISPR technology has witnessed a remarkable rise and success from the year 2010 up to date, in the year 2012, two scientist known as Jennifer Doudna and Emmanuelle Charpenteir shown CRISPR can be used to edit human cells outside the body, they reported the possibility of the combination of crRNA and the tracrRNA to create one synthetic guide to edit gene. Since then, so many scientists have used CRISPR to edit plant and animal genome. In the year 2015, Chinese scientist Junjiu Huang and his colleagues used CRISPR to modify nonviable human embryos Table 1.

Biogenesis of CRISPR

CRISPR are segments of DNA containing short repetitions of base sequences. Each repetition is followed by short segments of "spacer DNA" from previous exposures to a bacterial virus or plasmid Figure 1. Many Bacteria have in their cells adaptive immune system (a genetically vaccination card) called CRISPR that allow them to detect viral DNA and destroy it. When a virus infects a bacterial cell by injecting it's DNA, the bacterial CRISPR system allow the DNA to be inserted in a site called CRISPR, a mechanism that allow bacteria to record overtime the viruses they have been exposed to and those bits of DNA are passed on to cells progenies [8]. Upon another viral attack, the CRISPR DNA transcribes an RNA called a guide RNA, a complementary sequence to

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the target viral DNA which binds to a protein called Cas9 and form a CRISPR-Cas9 complex Figure 2. These chimeric complex searches in the cell to find a site that matches the sequences and cut it. Cells have the ability to detect broken DNA and repair it which sometimes causes mutation.

CRISPR technology

The technology allows scientist to manipulate the sequence of DNA in a precise manner. CRISPR-CAS9 system is a remarkable tool for greatly simplifying gene manipulation (Insertion, deletions, development of knock in and knock out of gene). A versatile genome-editing technology that is widely used for studying the functionality of genetic elements, creating genetic modified organisms and research for genetic disorders [9]. CRISPR technology is like a software for the genome, the Cas9 complex is programmable, scientist program the complex to recognise particular DNA sequence and cut the DNA at that site and later inserting a new DNA of interest (gene knock in), or cutting out a gene (gene knock out). This procedure is illustrated below Figure 3:

Advantages of using CRISPR technology: CRISPR technology is very simple, easy to use and cheap unlike the previous gene editing techniques such as Transcription activators-like effective nucleases (TALENS). This technology can be employed to analyse the interaction of genes and relationship between genetic differences and expression (phenotype). It can also be used to knock out gene and replaced it with another gene for diseases therapy [10].

Disadvantages of CRISPR technology: CRISPR-Cas9 off-target: The effect of off-target can alter the function of a gene and may result in genomic instability, hindering it prospective and application in clinical procedure. A Single guide RNA also known as chimeric RNA is the combination of CRISPR RNA (CrRNA) and trans-activating RNA (TrRNA). SgRNA's 20 base nucleotides are complimentary to the target DNA of interest with a trinucleotide known as Protospacer adjacent motif (PAM) adjacent to sequence which is mostly NGG (where N can be Adenine, Guanine, Cytosine or Thymine). Other studies reported that the PAM can also be NRG (where R is Adenine or Guanine) [11]. Even though DNA complimentary is highly specific based on base pair rule (A-T and G-C) there is still occurrence of off-target with 3 to 5 mismatches within the distal part of the PAM. SgRNA 5^' X_{20} PAM-3^' (5^' X_{20} NGG-3^') where X_{20} is CrRNA contain both non-seed and seed sequences (Figure 4) with many studies reporting that the seed sequence (mainly made up of 10-12 base pairs adjacent to the PAM) control the specificity of RNA guided endonucleases (RGens) and are regarded as more significance than the non-seed sequences [12].

Year	Scientist	Discovery
1987	Yoshizumi Ishino	Discovered CRISPR in E.coli
2000	Mojica and colleagues	CRISPR is present throughout prokaryotes and archaea
2002	Jansen et al.	Proposed the word CRISPR
2005	Mojica and colleagues	Sequence between repeat are surprisingly foreign
2007	Alexander Bolotin	Discovery of Cas9 and PAM
2007	Phillipe Horvath	Bacteria are immune to virus and stored a Viral DNA into their CRISPR
2010	Sylvain Moineau	Cas9 is guided by spacer Cas9 cleaves target DNA
2011	Emmanuelle Charpenteir	Discovery of tracrRNA for Cas9 system
2012	Charpenteir and Doudna	crRNA and the tracrRNA could be fused together to create a single synthetic guide for gene editing
2013		The Use CRISPR-Cas9 for genome editing in eukaryotic cells
2014	Nishimasu and Feng Zhang	Crystal structure of Cas9 gRNA complex

Table 1: History of CRISPR.

CRISPR gene editing with machine learning

Off target have been the major constraints of using CRISPR technology. Off targets of CRISPR where a wrong target sequence is moustached and hybridized is a great limitation for the application of CRISPR technology in several applications [13-15]. It was understood by researchers that CRISPR-Cas9 can only target 20 base pairs of sequences with Protospacer adjacent Motif (PAM) next to the pairs. But now some researchers have turned their attention to designing specific enzymes that will be able to recognise the PAM [16]. So many tools are designed to reduce off target effect such as Bowte alignment which only allows 3 mismatches and BWA tools ranging up to 5 mismatches. But still there is no tool efficient enough to solve off target effect. A lot of common off target tools can only calculate score base on the mismatch position to guide RNA [17]. So many computational method are employed for predicting both on target and off target effect, with some focusing on twenty base pair and some both the base pair and PAM. Both the on target and off target are incorporated into a mathematical design model with others including position of specific nucleotides, dinucleotides, Guanine and Cytosine content and Global Nucleotide Count (GNC). The trend procedure involve the inclusion of information regarding non-sequence effect such as thermodynamic stability of the guide RNA (SgRNA) and the endonuclease cut site position relative to start site of DNA transcription [18]. Scientist has gone forward to create different model such as Elevation [19], demonstrated how to increase on-target effect and reduce off-target effect on knockout gene using machine learning (ML). These techniques are used to improve the on-target effect efficiency using Azimuth model and to reduce off-target effect using an elevation model. Given a guide, these model filter genome and asses wide potential off-target, score each off-target from (1) for activity using a ML predictive model and aggregate the score from (2) into a single overall off-target score. Other scientist create linear regression model, Random Forrest Model and support vector machines. With all these models, there still remain a major constraint on the accuracy, precessions and predictions of both on target and off target effect with some highlighting on the method for transcribing RNA as one of the influence of the models prediction activity. The study shows each model work efficiently on the specific target use to create it. These models can be use by scientist but an advanced and efficient model need to be design to counter off target and increased off target effect [20].

The promise of designing a baby with CRISPR / CAS technology

Although ethics committee is still debating the risk that designing a baby may pose. Nevertheless, most parent in this generation would be excited with the possibility of giving birth to a child of accurate desirable traits such as heights, beauty, intellectual, physical appearance, eyes colour, skin type, hair type, amongst endless others. Although the use of CRISPR can be used to modified human gene, this research is not approved by gene editing governing bodies until all the challenges are solved and safety is adopted [21]. Regardless of all ethical debates and laws, a group of Chinese scientists have published a paper in April 2015 on the use CRISPR-Cas9 on human non-viable embryos. Their main objective was to find a cure for beta-thalassemia a hereditary blood disorder, but their experiment could not develop into human foetus. A year later another Chinese team use CRISPR-Cas9 on human

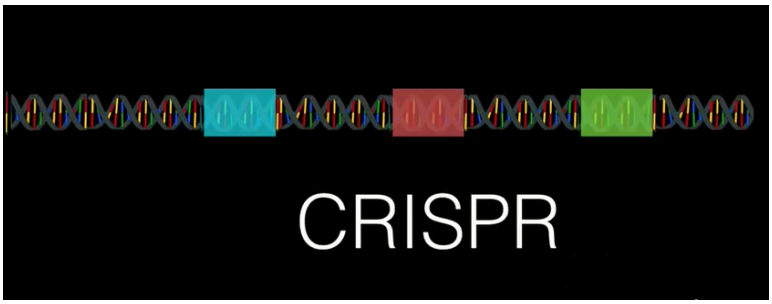

Figure 1: CRISPR (The blue, red and green colour indicate different Viral DNA sequences store in the Bacterial).

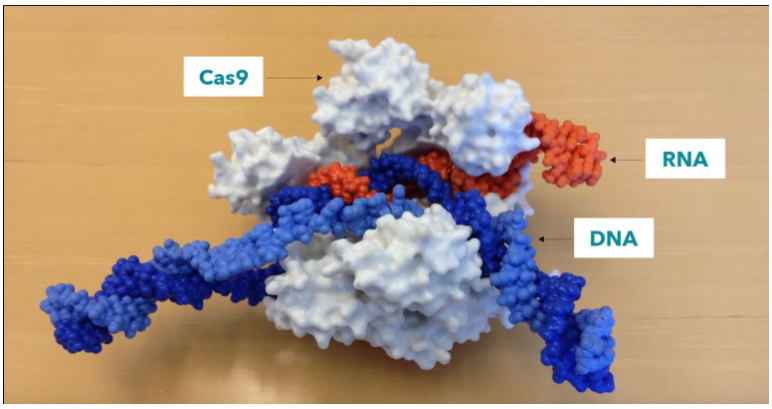

Figure 2: Combination of Cas9 protein, a transcribed CRISPR RNA and new Viral DNA (Doudna et al. 2017).

zygote to induce mutation on genes to make zygotes immune to HIV Virus. In 2016 these techniques are used to engineer human immune system to fight against Cancer [22]. A poll was conducted in USA by National Academy of Science (NAS), Harvard School of Public Health and International Summit on Human Gene Editing and more than 80% were not in favour of using CRISPR-Cas9 technology to improve babies trait such as intelligence and physical appearance of unborn babies [23] highlighted three problems concerning the use of CRISPR on germline gene, first, it can create high risk for the baby, secondly, the need for illicit techniques and lastly it can create risk for human beings by introducing new changes to the genes Figure 4.

Application of CRISPR-CAS9 in agriculture

Due to increase of population and high demand of food worldwide, It is reported as a result of changes of climate and global warming there will be high increase of poverty and food shortage. So many technologies have emerged such as sequence specific nucleases (SSNs), transcriptional activators-like effector nucleases (TALENs) and Zinc Finger nucleases (ZFNs) to increase and improved yield [24]. The emergence of CRISPR-Cas9 has shown greater promise in improving crop yield and preventing crop genetic diseases. This technology can be employ to modify plant genome [25]. The application of this new and trending technology is increasing rapidly with the aim of developing non-transgenic genome edited plant to avoid adverse changes that may occur as a result of climate change. CRISPR-Cas9 has a promise on plant to a new level such as modifying plant to adapt to changes in climate and tolerance to harsh condition, less disease and improved crop quality and yield [26]. Scientist has made so many advances in editing plant gene using CRISPR-Cas9 technology. A group of researchers from cold spring harbour (CSH) worked on tomato by engineering quantitative gene promoter sequence, this approach led to positive changes in the tomato's shape, size and architecture. Another research on rice has shown increased in seed size, another study on *Brassica napus* has shown increased in seed number and grain weight. Different type of CRISPR-Cas9 vector were developed which result in more efficient way of editing plant genomes by either knocking out a gene, deletion of gene, disruption of cis-regulatory element and decreasing virus diseases [27].

Other application of CRISPR technology

Another current application of this technology is the introduction of deleterious genes into malaria carrying mosquitoes, one of the ways to tackle malaria diseases. Scientist in Philadelphia United states of America has shown they can remove DNA of integrated HIV virus from infected human cells a hope in the pursuit of HIV-AIDS disease. The future for the use of CRISPR technology is unlimited, it cannot be quantified but can only be predicted; According to Doudna et al 2017, CRISPR technology is aimed to help treat cancer, to engineer human being that has strong bones, less susceptible to cardiovascular diseases, or to design human with specific trait and for Parent to pick babies sex, eyes, height and another desirable trait. Scientist has devoted researches on Anti-ageing, hoping with these technology human beings' live expectancy will be above 100 years.

Limitations of CRISPR technology

One of the challenges of using CRISPR-Cas9 editing technique is off-

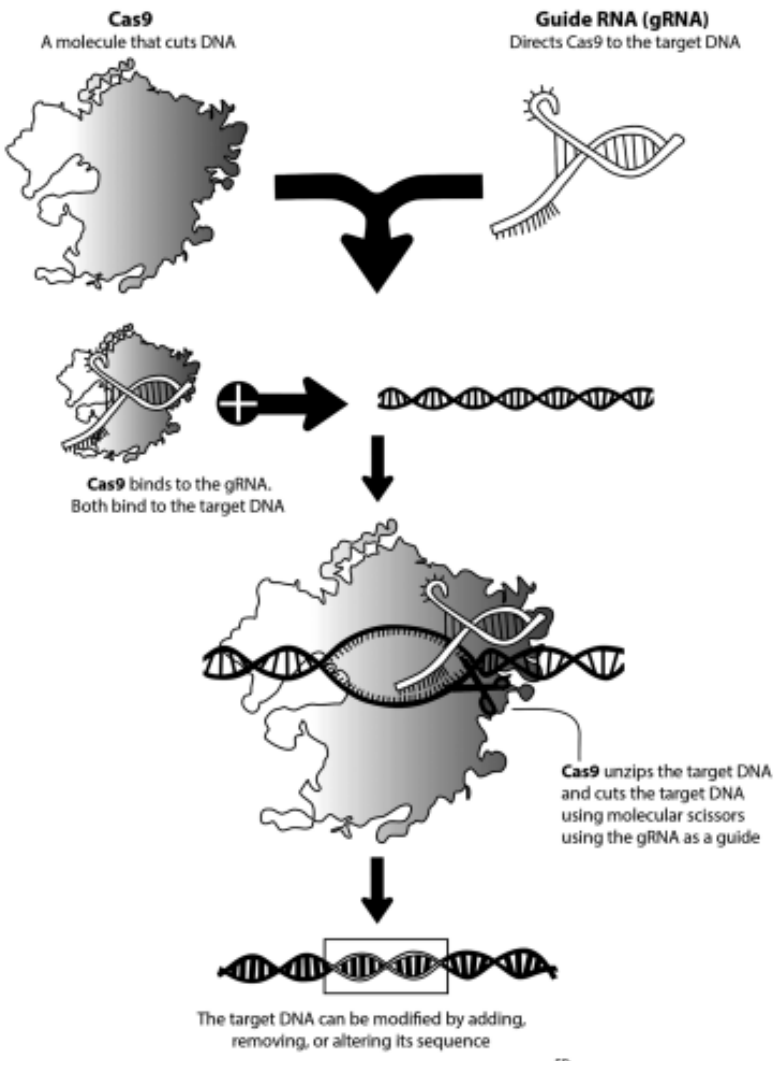

Figure 3: Procedure, Principle and mechanism of CRISPR technology (Scherz P 2017).

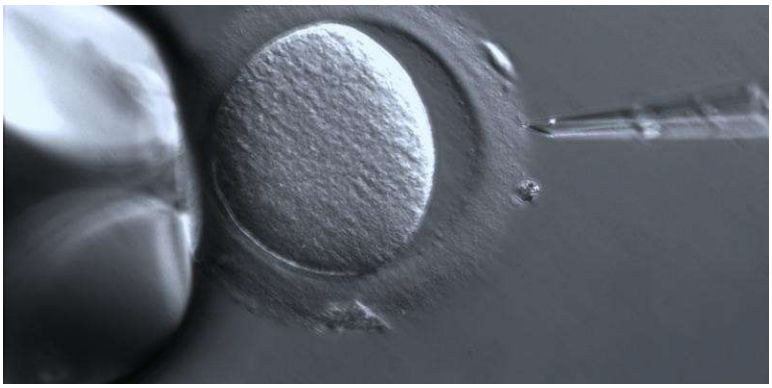

Figure 4: Embryo Injection on CRISPR (ZEISS Microscopy, CC BY-SA URI, 2018).

target effect where Cas9 enzymes cut wrong genes. Targeting specificity of Cas9 is known to be tightly controlled by twenty nucleotide guided sequence of the SgRNA and the presence of protospacer adjacent motif (PAM) next to the target sequence in the gene, potential off target cleavage activity could still occur (50% chance) on DNA sequence with even 3-5 base pair mismatches.

Conclusion

CRISPR Technology as a tool for gene editing has shown to be a saviour for human being's health diseases especially genetic diseases that can be pass from parents to offspring. The possibility of genetically designing a baby is very near with the technology. However, as advantageous as the technology is, it has its own challenges such as off target, a process where Cas9 complex cut at undesirable site, thereby requiring further research to reduce this hinderance. Furthermore, the use of this technique needs a legal regulation in order to avoid engineering organism that may pose treats to humans, animals, plants and microbes.

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