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A review on CRISPR/CAS9 system tool for genome engineering

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Abstract
The recently developed genome engineering techniques depend on CRISPR/Cas9 are revolutionizing the field of life science. CRISPR was first described in Escherichia coli in 1987. The CRISPR-Cas9 is mainly derived from the type II CRISPR-Cas systems. The new development of the CRISPR-Cas9 technology is purposeful including a simple-to-use editing tool. CRISPR/Cas9 is a newly developed useful technology in genome editing of excellent advantages. This is majorly contributed to life science fields like plant breeding, animal breeding, and medicine. The CRISPR/Cas9 is widely used in agriculture like enhanced yield, increase crop disease resistance as well as improve crop quality. CRISPR/Cas9 is used in the treatment of various human diseases. CRISPR/Cas9 is rapidly developing in the area of molecular biology and improved gene therapies. The CRISPR/Cas9 is provided great advances in cancer immunotherapy and pancreatic cancer.

Keywords: CRISPR/CAS9, genome engineering, gene therapy

Introduction
CRISPR refers to the clustered regularly interspaced short palindromic repeats that are most essential for the life sciences and have rapidly increased in recent years [1]. The CRISPR was first discovered in Escherichia coli in the 1987 year-like sequence of repeated segments of 29 nucleotides (nt) in the length interspaced to the different sequence segments of 32 nucleotides. The CRISPR system is related to Cas genes showed that described of same small-repeated palindromic sequences of 20-40 nucleotides in various classes of bacteria as well as archaea [2]. CRISPR-Cas9 system is mainly derived from the type II systems [3]. The CRISPR/Cas9 system in which involved the two essential components such as Cas9 as well as sgRNA. The Cas9 is the DNA endonuclease and is mainly obtained from several bacteria including Streptococcus thermophilus, Streptococcus pyogenes, Brevibacillus laterosporus, and Staphylococcus aureus. The Cas9 is mainly isolated from the Streptococcus pyogenes. The Cas9 is consist of two domains like HNH domain as well as RucV-like domain. The sgRNA is the synthetic RNA and the length is around 100 nucleotides (nt) [4]. The newly developed genome engineering technologies are majorly dependent on the category of RNA-guided endonucleases, like CRISPR-related Cas9 [5]. Genetic engineering is the precise technology it refers to the modification of genes at the molecular extent [6]. Genome editing is an important part of genetic engineering that modifies a particular genome inappropriate and predictable way [7]. The CRISPR/Cas9 technology is an important tool of genome editing. This is an improving development and various applications; this is the essential system for precise and potent genome editing [8]. The CRISPR-Cas9 technology is a genome-editing tool that has more than a few importance. They are greatly participated in the area of life science, in addition to medicine, animal breeding as well as plant breeding. CRISPR-Cas9 is widely useful for the study of several genetic diseases. The CRISPR-Cas9 is also used in crop development that is enhanced disease resistance, nutrition improvement as well as increased yield [9]. The CRISPR-Cas9 technology is also useful for several other aspects like labeling for live cells of chromosomal loci, regulating endogenous gene expression. The CRISPR/Cas9 is a tool of gene editing that provides progressive development in the area of molecular biology as well as improved gene therapies [10]

Components of CRISPR/Cas9
The CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats [11]. The CRISPR is the clustered family of short repeats of the DNA these make an integral part of the bacteria as well as archaea adaptive immunity [12]. CRISPR/Cas9 was first described in Escherichia coli in the year 1987 [13]. The CRISPR systems are mainly classified into two essential classes class I involved (type I, III, I) as well as class II consists of (type II, V, VI) [14]. Class I systems involved multiple subunits of Cas-protein complexes and other class II systems used only one Cas-protein [15]. The type II CRISPR/Cas9 structure is comparatively simple, that are studied very well and greatly useful in genetic engineering. The Single Guide RNA (sgRNA), as well as CRISPR-related (Cas-9) proteins these two main components of the CRISPR/Cas9 system [16]. The cas-9 protein is a first Cas protein that is mainly useful in genome editing that is majorly isolated from the bacteria Streptococcus pyogenes (SpCas-9). Those are high (1368 amino acids) and the multiple domain DNA

endonucleases are mainly helpful for cleaving the specific DNA and then obtaining a double-stranded break that is known as genetic scissor [17]. The Cas-9 in which involved two main regions such as the recognition (REC) lobe as well as the nuclease (NUC) lobe. The REC lobe involved a REC1, as well as REC2 domains, is mainly useful for binding guide RNA. Another is the NUC lobe which mainly consists of Protospacer Adjacent Motif (PAM) interacting, HNH as well as RuvC domains [18]. The single guide RNA (sgRNA) is formed from two parts (crRNA) and the other is (tracrRNA) [19]. The crRNA is composed of 18-20 bp in length which specifies a particular DNA and then forms a pairing to the target sequence. The tracrRNA is an elongated stretch of the loops which are useful for binding of scaffold for the Cas-9 nuclease [17].

CRISPR/Cas9 Mechanism

The adaptive immune system of CRISPR/Cas9 involved the three stages such as adaptation, expression as well as interference. The adaptation phase consists of invading the DNA from the virus as well as plasmids these are cleaved into small segments and inserted in the CRISPR locus. The CRISPR loci transcripts are processed to form the multiple CRISPR (cr) RNAs using the tracrRNA in the crRNA biogenesis. The DNA interference in which Type II of CRISPR/Cas system needs only one Cas9 protein [20]. Cas9 is a large protein having multi-domains (The RuvC domain is present at the amino-terminal and the other is HNH nuclease domain is located in the middle and the two main RNAs such as CRISPR RNA as well as trans-activating crRNA [21]. The interference in which Cas9 endonuclease is complexed to the crRNA and cleaved a foreign DNA close to the PAM region [20].

New Variants of CRISPR System

The studies which described the enzymes associated with the CRISPR family, consist of enzymes that are encoded the Cas genes smaller than the SpCas9 (4.2 kb), like SaCas9 (3.2 kb), St1Cas9 (3.4 kb), as well as NmCas9 (3.2 kb). Those enzymes would promote its packaging to the viral vectors. The other recently described enzyme known as Cpf1 with the smaller crRNA sequences is used alternative to SpCas9. Furthermore, the recently identified C2c2 (Cas13a) as well as C2c6 (Cas13b) that are cleaved the RNA [22].

Common techniques in genetic engineering

The genetic engineering process is the direct manipulation of organisms or populations of organisms with the help of the recombination of DNA [23].

Recombinant DNA

Recombinant DNA (rDNA) is a form of DNA created in the laboratory of genetic recombination [24]. Recombinant DNA molecules can be produced by the combining of two or more distinct strands of DNA, scientists can produce a new strand of DNA [25]. Recombinant DNA was first started in 1970 by Paul Berg of Stanford University. In 1973 the scientist Herbert Boyer of the University of California (San Francisco), used E. coli who invented developed the recombinant human insulin by Eli Lilly company in 1982 [26]. Recombinant DNA technology is used to give a particular development in several specialties it consists of gene therapy, crop agriculture, vaccine design, and pharmaceutics as well as for bioremediation [27].

Microinjection

This is a technique of genetic engineering. In which inject a substance into the cell with the help of a small glass micropipette. In the process of microinjection DNA as well as another genetic substance introduced in a cell, the gene is transferred more effectively giving rise to gene expression and the integration in the host cell genome. The two main applications of the microinjection technique such as to form transgenic animals as well as in the area of in vitro fertilization [28].

Bio ballistics

This is also a technique of genetic engineering. In which use of metal silvers coated to appropriate gene. The metal silvers that are small in size generally smaller than the cell together with a proper genetic material are introduced into the shotgun. That shotgun is a targeted cell of interest and then in which introduced the genetic material. After the proper genetic material is entered in the target cells, then it searches the nucleus and invades into it, and in that combined to the genes of the host cell then appear appropriate characteristics [29].

Electro and Chemical Poration

It is the technique of genetic engineering in that cells are formed a porous for that gene are enter into it. Pores are produced in the cells to bathing it in particular chemicals or to bathe the cells through the use of electric current. With the help of pores genetic material is entered in the cells and then finds a nucleus and enter into it and In the nucleus genetic material is combined to the genes of host cell then appear appropriate features [29].

Genome Engineering

Genome engineering is the most useful technology in the field of biological research ad well as biomedical importance, that is carried out to Changed the genomic sequences that are to regulate the basic genetic information in the cell. Genome engineering in which the use of engineered nucleases is highly designable as

well as a scalable way to attain specific editing of the genomic sequences [30]. There are mainly three types of genome engineering methods such as ZFNs, TALENs including the CRISPR-Cas system. The ZFNs refer to the Zinc-finger nuclease which is formed by the combining of zinc finger DNA- domain with DNA-cleavage domain [31]. The TALENs stand for the Transcription activator-like effector nucleases it is a fusion protein, it involved the DNA-binding domain as well as the DNA-cleavage domain. This DNA-cleaves domain is similar in the ZFNs as well as TALENs (Catalytic part of FokI) and DNA binding domains are distinct. TALEN DNA-binding domain that mainly obtained for the TALE proteins that occurred in Xanthomonas plant pathogen. The CRISPR/Cas system is an adaptive immune system generally found in the bacteria as well as archaea that are opposed to invaders plasmids as well as viruses. Type II system is mainly acquired as genome engineering a few times earlier. This system involved two essential components such as Cas9 protein including the RNA complexes it involved of (crRNA) as well as (tracrRNA) [32].

CRISPR/CAS9 system in Genome Engineering

Genome engineering is a precise improvement over genetic engineering in trying to attain a high particularity on the target, flexibility as well as adaptability of the gene editing and it determining the capability off-target effects. Genome engineering used engineered nucleases is a highly designable as well as expandable way of attaining specific editing of the genomic sequences [33]. The newly developed genome engineering techniques mainly depend on CRISPR/Cas9 and they play important role in the various life science field. The CRISPR/Cas9 is mainly derived from the type II system that advances sequence-particular nuclease for the option to the genome engineering due to the three main purposes. 1) The Cas9 is guided to the single guide RNA (gRNA) which is simply engineered. gRNA target sequence presents 20 nucleotides (not), that is similar to a DNA target position and it could be ordered like pair of the oligonucleotides and fastly cloned; 2) The CRISPR-Cas9 is modular characteristics and the small 20 nucleotides (nt) in the length of targeted gRNA formed that components useful for the capacity to target and that are cleaved the many target sequences at the same time (multiplexing); 3) CRISPR-Cas system is capability, as well as high selectivity with the less off-target effect of the undesired chromosomal translocations at designed gRNA, is useful [33, 34].

Applications of CRISPR/Cas9

CRISPR is a rapidly developing technology that is majorly useful for life sciences. The CRISPR technologies are majorly useful for plant breeding, disease modeling, animal breeding as well as biotherapy [35]. The CRISPR/Cas9 is too useful to enhance crop quality and increase crop disease resistance [36]. The recent advancement in CRISPR/Cas9 technology has increased the capability for proper genome manipulation. CRISPR/Cas9 is shown remarkable progress in basic biology, biotechnology, and the fields of medical research [37].

CRISPR-Cas9 in Gene therapy

Gene therapy is the process in which replacing the defective genes with the correct gene. The rapidly developing CRISPR/Cas9 system is useful for gene therapy to recover from its stigma and also enhance the therapeutics aspects [38]. The CRISPR/Cas9 system is majorly used in treating various genetic diseases. The targeted gene correction is the capability to treat several diseases, involved clotting disorders like hemophilia A and B, Fabry disease, muscular dystrophy, Gaucher disease, von Gierke disease, cystic fibrosis, Pompe disease, Hurler as well as Hunter syndromes [39].

CRISPR/Cas9 in Cancer Immunotherapy

Cancer immunotherapy is a great advance in the field of biomedical research [40]. Immunotherapy is the developing therapeutic technique with rising clinical results in tumors. The one perspective used in immunotherapy an immune checkpoint inhibitors like blockage of CTLA-4, PD-1, including their ligand PD-L1. The majority of study gives the CRISPR-Cas9-intervented PD-1, PDL-1 and CTLA-4 gene knockout is efficient action that breaks tolerance to T-cell from adoptive therapy in the tumor therapy. The two main trails are in the CRISPR/Cas9 system that engineers T cells of the cancer patients to inactivate the PD-1 is simply in the process [41].

CRISPR/Cas9 in Pancreatic Cancer

CRISPR/Cas9 system is mainly useful for the research in pancreatic cancer that knocked out the genes involved in the disease development. The CRISPR/Cas9 is used that knocked out KDM6A in the human pancreatic ductal adenocarcinoma (PDAC) cell lines to determine KDM6A- lacking cells show an aggressive physical composition. CRISPR/Cas9 is used to knock out the particular genes and described the phenotypic alterations, it describes the good perception of the biological functions act by the target genes, determine probable treatment targets, as well as a good treatment method for pancreatic cancer. The CRISPR/Cas9 dependent gene therapy consists of two techniques like ex vivo as well as in vivo. The ex vivo method takes place outside of the organism. The in vivo method carrying out in the living organism. The creation of in vivo pancreatic cancer models by retrograde pancreatic ductal injection of either adenoviral-Cre as well as lentiviral-Cre vectors. That method is formed a recent treatment for pancreatic cancer. CRISPR/Cas9 plays an important role in pancreatic

cancer research and is described as the "game-changer" in scientific study and it is an interesting targeted therapy to pancreatic cancer [42].

CRISPR-Cas9 in Agriculture

CRISPR/Cas9 is a new gene-editing technology that plays a great role in the agriculture field. The CRISPR/Cas9 is used for increasing the resistance against crop diseases and they enhance tolerance to main abiotic factors like drought as well as salinity [43]. The CRISPR/Cas9 is mainly useful for enhancing crop quality. This technology is mostly useful for crop improvement [44].

Conclusion

The best Mechanism to alter, modify and regulate the genomes is CRISPER-Cas Mediated Genome Engineering. CRISPER-Cas System is a great evolution in the Molecular biology field that has broad applications in various streams like analysis of human and animal genome function, Gene Immunotherapy, Pancreatic Cancer therapy, in some cases, this system is used to visualize the specific region of DNA, like telomeres. CRISPR/Cas9 technology involves lower costs, greater efficiency, and ease of use. But there are some limitations for the revolutionary CRISPER-cas system which need to be overcome in the future for Significant safety and long-term efficacy.

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