CRISPR-Cas Based Genome Editing in Plants: Recent Advances and Future Prospects

Abstract

The CRISPR/Cas system has emerged as a powerful tool in agriculture and plant breeding, offering innovative ways to improve crop traits and address various challenges in food production. It enables precise and targeted genetic modifications, allowing researchers to enhance crop characteristics, increase yield, improve nutritional value, and confer resistance to pests, diseases, and environmental stresses. One of the key applications of CRISPR/Cas in agriculture is the development of disease-resistant crops. By targeting and modifying specific genes responsible for susceptibility to diseases, plants can be engineered to possess enhanced resistance, reducing the need for chemical pesticides and promoting sustainable farming practices. CRISPR/Cas also facilitates the improvement of crop traits such as yield and nutritional content. Researchers can target genes involved in plant development, flowering time, fruit quality, and nutrient uptake to enhance these traits and produce crops with improved productivity and nutritional value. For instance, the system can be used to increase the vitamin or mineral content of crops, addressing malnutrition and improving human health. Another valuable application of CRISPR/Cas in plant breeding is the development of drought-tolerant and climate-resilient crops. By modifying genes associated with water use efficiency and stress response, crops can be engineered to withstand drought, extreme temperatures, and other environmental challenges, ensuring

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stable yields and minimizing losses due to climate change. One significant advantage of CRISPR/Cas in agriculture is that it allows for precise genetic modifications without introducing foreign genes from unrelated organisms. This means that crops developed using CRISPR/Cas are often considered non-transgenic or non-genetically modified organisms (GMOs), which can ease regulatory hurdles and public acceptance. However, it is important to note that the regulation of genetically modified crops varies across countries and regions. The use of CRISPR/Cas in agriculture and plant breeding may be subject to specific regulations and guidelines that differ from those governing traditional breeding methods.

Keywords: *Genome editing, CRISPR/ Cas, precise, target specific, efficient editing*

I. CRISPR/CAS- AN INTRODUCTION

The CRISPR/Cas system is an adaptive immune system adopted from archaea/bacteria, comprising of CRISPR repeat-spacer arrays and Cas proteins, accidentally discovered in 1987 in E. coli. The CRISPR–Cas systems mediates immunity to invading genetic elements via a three-stage process-adaptation, expression and interference. At the adaptation stage, a distinct complex of Cas proteins binds to a target DNA, often after recognizing a distinct, short motif known as a protospacer-adjacent motif (PAM), and cleaves out a portion of the target DNA, the protospacer. After duplication of the repeat at the 5′ end of the CRISPR array, the adaptation complex inserts the protospacer DNA into the array, so that it becomes a spacer (Kumar *et al.*, 2016). Some CRISPR–Cas systems employ an alternative mechanism of adaptation-namely, spacer acquisition from RNA, via reverse transcription by a reverse transcriptase encoded at the CRISPR–Cas locus. At the expression stage, the CRISPR array is typically transcribed as a single transcript - the pre - CRISPR RNA (pre-crRNA), that is processed into mature CRISPR RNAs (crRNAs), each containing the spacer sequence and parts of the flanking repeats. In different CRISPR–Cas variants, the pre- crRNA processing is mediated by a distinct subunit of a multiprotein Cas complex, by a single, multidomain Cas protein, or by non- Cas host RNases. At the interference stage, the crRNA, which typically remains bound to the processing complex (protein), serves as a guide to recognize the protospacer (or a closely similar sequence) in the invading genome of a virus or plasmid, which is then cleaved and inactivated by a Cas nuclease (or nucleases) that either is part of the effector or is recruited at the interference stage (Barrangou, 2013; Sharma *et al.*, 2023).

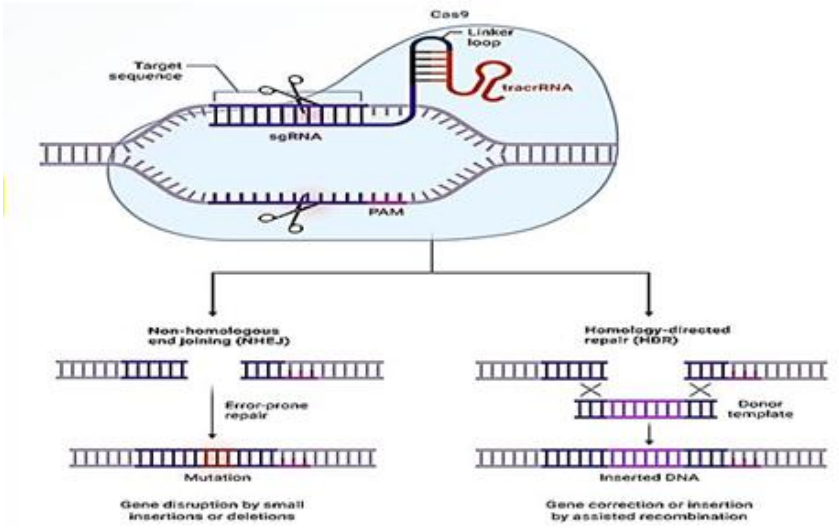

Figure 1: Streptococcus adaptive immune response against viruses.

Adapted from "2020 Nobel Prize in Chemistry: A Tool for Genome Editing (CRISPR-Cas9)", by BioRender.com (2020) https://www.bio-rad-antibodies.com/blog/how-crispr-revolutionized-science.html

II. CLASSIFICATION OF CRISPR-CAS SYSTEM

Based on Cas genes and the nature of interference complex, RNA mediated CRISPR/Cas system is divided into two classes, which have been subdivided again into six types. Class 1 CRISPR/Cas systems include type – I, III and IV, which accomplishes multi- Cas protein complexes for their interference and class 2 CRISPR/Cas systems includes type – II, V and VI, that do not employ multi-Cas proteins, instead single effector proteins along with CRISPR RNAs (crRNAs) are employed. Among all the types, type II was well studied and it was the first system that specifically cleaves DNA in vivo and in vitro derived from Streptococcus pyogenes (Viana *et al.,* 2019). CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/CRISPR-associated protein 9 (Cas9) consists of two small RNAs- the CRISPR-RNA (crRNA) and the trans-activating crRNA (tracr RNA), which directs the nuclease (Cas9) to a specific DNA sequence complementary to the crRNA. These two components have been re-engineered into a more manageable single element called the guide-RNA (gRNA), which includes 20 nucleotides complementary to the target region (Kumar *et al.,* 2019). Cas9-gRNA will recognize the target region immediately upstream of PAM sequence (Protospacer Adjacent Motif, mostly 5'-NGG-3'). After recognition of the target sequence, Cas9 protein cleaves the DNA, 3 nucleotides upstream of the PAM on the complementary strand and 3–8 nucleotides upstream on the non-complementary strand. It generates a Double-Stranded Break (DSB) in the genome at the target loci and the endogenous DNA repair machinery will take up the repair process via either non-homologous end joining (NHEJ) or homologous recombination (HR) pathway (Shan *et al.,* 2014). NHEJ repairs the double stranded breaks in an error-prone manner leading to loss of function mutants (knock out), whereas HR pathway repairs by employing a DNA template leading to gain of function mutants (knock in).

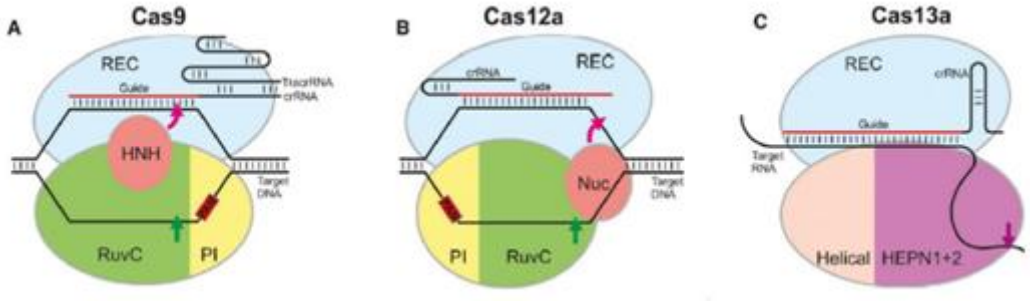

Figure 2: Classification of CRISPR- Cas system (Schindele *et al.,* 2018)

2.1 Cas9

2.1.1 Cas9 as a Nuclease (A)

The CRISPR/Cas9 system mediates its function via the single effector Cas9 and two small RNAs, the crRNA and tracrRNA. Upon hybridization, the crRNA, tracrRNA complex associates with the Cas9 nuclease and binds to its recognition site upstream of the PAM sequence. DNA binding is mediated by the 20-nucleotide guide sequence of the crRNA. The Cas9 nuclease induces a blunt-ended DSB 3 bp upstream of the PAM (NGG) sequence. Recognition of the crRNA, tracrRNA, target complex is mediated by the REC (recognition) lobe, the PI (PAM interacting) domain is responsible for PAM recognition. The DSB is mediated by the HNH and RuvC nuclease domains, with the HNH domain cleaving the target and the RuvC domain cleaving the nontarget strand (Steinert *et al.,* 2015).

2.1.2 Cas9 as a Nickase

The Cas9 enzyme comprises two nuclease domains for DSB induction, namely the HNH and RuvC domain, each cleaving one strand of the target DNA. By introducing a point mutation into the catalytic site of the HNH or RuvC domain, the Cas9 enzyme can be converted into a nickase. Paired nickases can be used to reduce off-target activity, where the Cas9 nickase is guided by two sgRNAs, each binding one strand of the DNA in close proximity in such a way that a DSB with 50-overhangs is generated. As individual single- strand breaks (SSBs) at potential off-targets do not activate error-prone repair via NHEJ, off-target mutagenesis is decreased. The programmed conversion of single bases without the induction of DSBs by targeting deaminases to the site of interest can be achieved by either fusing a cytidine deaminase or an adenosine deaminase to the Cas9 nickase (conversion of C/G to T/A or A/T to G/C) (Komor *et al.*, 2016).

2.2 Cas12a (formerly named Cpf1)

2.2.1 Cas12a as a nuclease (B)

The CRISPR/Cas12a system mediates its function via the single effector Cas12a and a single crRNA. Upon association of Cas12a and crRNA, the complex binds to its recognition site downstream of the PAM sequence. DNA binding is mediated by a 23–25-nucleotide guide sequence of the crRNA. The Cas12a nuclease induces a staggered-ended DSB distal from the PAM sequence. Recognition of the crRNA, target complex is mediated by the REC (recognition) lobe, the PI (PAM interacting) domain is responsible for PAM (T-rich) recognition. The DSB is mediated by the Nuc and RuvC domains, with the Nuc domain cleaving the target strand after the 18th bp downstream of the PAM and the RuvC domain cleaving the nontarget strand after the 23rd bp downstream of the PAM (in case of FnCas12a).

To enable the expression of multiple guides from a single transcript, ribozyme- or tRNA- based systems were developed for Cas9. In natural mechanism, the tracrRNA as well as RNase III activity are required for the processing of the pre-crRNAs. For Cas12a, it was shown that the enzyme itself processes the pre-crRNA using a catalytic site exhibiting endoribonuclease activity. Therefore, it naturally enables multiplex gene editing with a single array (Gao *et al.,* 2016).

2.3 Cas13 (Formerly Named C2c2)

2.3.1 Cas13 as a Nuclease (C)

The CRISPR/Cas13 system (Cas13a) mediates its function via the single effector Cas13 and a single crRNA. Upon association of Cas13 and crRNA, the complex binds to its recognition site within the target RNA mediated by the guide sequence of the crRNA. The catalytic site is located at the outside of the protein facing the surrounding solution, leading to cleavage of the target RNA remote from the recognition site. Recognition of the crRNA, target complex is mediated by the REC (recognition) lobe, cleavage of the target RNA by the HEPN domain. Two HEPN domains are located on the external surface of Cas13. As a dual ribonuclease, Cas13a can cleave pre-crRNA to generate crRNA maturation, and the helical-1 domain in LshCas13a and the HEPN2 domain in LbuCas13a are likely involved in pre- crRNA processing. LshCas13a-crRNA duplex could cleave other ssRNA in a non-specific manner once activated by target ssRNA, which is referred to “collateral effect” (Schindele *et al.,* 2018).

III. CRISPR/CAS9 MEDIATED GENOME EDITING IN PLANTS

CRISPR/Cas9 system has high target specificity, since Watson and Crick model of target site recognition is seen and the sequence analysis helps in identification of off-target sites (Zischewski *et al.,* 2017). CRISPR/Cas9 based genome editing can be used for plant functional studies and plant improvement to yield, quality, and tolerance to environmental stress, which overcomes the problems of time consumption, the requirement of large area for growing encountered by traditional breeding techniques (Demirci *et al.,* 2018; Kiran *et al.,* 2023). The major advantage of CRISPR/Cas system is that the possibility of multiplex genome editing with high precision, accuracy and high versatility.

The key step involved in genome editing is the delivery of the editing reagents into the plant cell and the production of targeted editing events. The transient expression can be accompanied with protoplast transfection, whereas the stable expression is necessary for the heritable modifications in the genome, which could be made possible by *Agrobacterium* mediated transformation (Zhang *et al.,* 2020). Since elimination or rejection of negative or inferior elements is a promising strategy for genetic improvement, knocking out the genes harbouring undesirable traits is the most common application of CRISPR/Cas9. The traits that have been improved using CRISPR/Cas9 includes yield, biotic and abiotic-stress resistance, quality etc. Recently, hybrid-breeding techniques also become a part of this tool.

Li *et al.,* (2013) was the first to demonstrate the CRISPR/Cas system of genome editing in model plants, *Arabidopsis* and tobacco. Woo and his team demonstrated the CRISPR/Cas9-mediated genome editing in rice, *Arabidopsis*, lettuce and tobacco. They used protoplasts as explants for their polyethylene glycol-mediated RNP transfection and achieved targeted mutagenesis at frequencies up to 46% (Woo *et al.,* 2015).

Low-gluten wheat has been created by knocking out the conserved domains in α-gliadin gene family (Sanchez-Leon *et al.,* 2018). Other crops produced with high-quality by targeted mutagenesis of CRISPR/Cas9 includes tomatoes with long shelf-life (Ito *et al.,* 2015) and enhanced γ-aminobutyric acid content (Li *et al.,* 2018a) or lycopene (Li *et al.,* 2018b) in tomato or high oleic acid content in seeds of *Camelina sativa* (Jiang *et al.,* 2017) and *Brassica napus* (Okuzaki *et al.,* 2018; Kumar *et al.,* 2024), and reduced steroidal gylcoalkaloids in potato (hairy roots) (Nakayasu *et al.,* 2018). Genome editing is also employed to enhance various traits like shortening growth times/early maturing cultivar development (Li *et al.,* 2017), improving haploid breeding (Yao *et al.,* 2018),

overcoming self-incompatibility in diploid potato (Ye *et al.*, 2018), and enhancing silique shatter resistance in *Brassica napus* (Braatz *et al.*, 2017). CRISPR/Cas9-mediated genome editing resulted in the development of viral free plants such as cotton leaf curl disease resistant (clcud) cotton (Iqbal *et al.*, 2016), and broad potyvirus resistant (*eif4e*) cucumber (Chandrasekaran *et al.*, 2016). The traits that were successfully improved using CRISPR were listed in Table 1.

IV. WHY CRISPR/ CAS OVER OTHER GENOME EDITING TOOLS

The three main types of genome-editing tools are zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the CRISPR/Cas system. ZFNs and TALENs have been utilised to carry out a range of genetic alterations by generating DNA double-strand breaks, which encourage error-prone nonhomologous end joining (NHEJ) or homology-directed repair (HDR) at certain genomic loci. The DNA cleavage domain of the restriction enzyme FokI and zinc-finger-based DNA recognition modules make up the ZFN family. Before joining together to cling to particular DNA sequences, each zinc finger locates and bonds to a nucleotide triplet (Ahmad, 2022). To create TALENs, the FokI restriction enzyme is coupled with the transcriptional activator-like effector (TALE) repeats (Hua *et al.*, 2022; Kumar *et al., 2022*; Randall *et al.*, 2022). Adenine (A), cytosine (C), thymine (T), and guanine (G)/adenine (A) are just a few examples of repetition variable residues (RVDs) and hypervariable amino acids at the 12^{th} and 13^{th} positions that were discovered using four different amino acid RVDs (NI, HD, NG/HG, and NN), which make up the core domain of TALE in this genome editing technology. The Cas9-CRISPR system is currently a well-liked and efficient gene-editing technique when compared to ZAFN and TALN. As a component of the bacterial adaptive immune system, CRISPR sequences may recognise foreign viral DNA and instantly cleave Cas proteins (Zetsche *et al.*, 2015; Kim *et al.*, 2016; Komor *et al.*, 2016). Despite the Cas9-CRISPR system's success, different Cas-9 proteins have been identified and applied to genome editing technology. In addition to Cas9, Cas12a/Cpf1 is the most well- known Cas protein; it can distinguish between various PAM sequences and generate sticky ends as opposed to blunt ends, making HDR correction simpler and resulting in more accurate editing. Some of the Cas enzyme subtypes include Cas3, Cas10, Cas12, Cas12b, Cas13a and CasF. The possibilities available to different plant kinds may be increased by these various enzymes (Kumar *et al.*, 2022). Modern genome editing technologies have altered the practise of synthetic biology. Since the middle of the 1990s, when molecular biology first emerged, technologies like CRISPR/Cas-based gene

editing have offered a number of ways to modify genomes and helped plant scientists achieve their goals. This method has been widely applied during the past seven years in practically every sphere of life, including the environment, healthcare, and agriculture. Scientists are currently engaged in more ambitious genomics studies to address the difficulties of food security in the wake of the expanding population and the hazards posed by climate change after successfully using gene editing to alter fundamental features (Ahmad *et al.*, 2021; Aslam *et al.*, 2021; Khan *et al.*, 2021; Ashraf *et al.*, 2022; Wang Z *et al.*, 2022).

V. RECENT ADVANCES

Recent experiments focus on modifying the elements in CRISPR/ Cas to enhance the precision and accuracy. New tools such as the (spCas9-NG, xCas9, Cpf1, Cas13, Cas14, base editing, prime editing etc., are gaining attention in the area of genome editing. Recent research has shown that SpCas9-NG, a SpCas9 variant rationally developed from the SpCas9-sgRNADNA complex structure, can recognise relaxed NG PAM sequences. xCas9 and SpCas9-NG both recognise laxer PAM sequences in comparison to SpCas9. While xCas9 and SpCas9- NG variants that recognise relaxed NG PAMs can work better in rice, SpCas9- NG is better suited for base editing in rice. Compared to SpCas9, xCas9 often demonstrates more DNA specificity and editing effectiveness, reduced off-target activity, and improved PAM compatibility (Aslam *et al.*, 2021; Khan *et al.*, 2021; Ahmad, 2022; Ashraf *et al.*, 2022; Ahmad, 2023). Both tracr RNA and crRNA are required for Cas9 to be recruited. Tracr RNA is not required for the CRISPR/Cas12a system since the Cas9 then causes a DSB, producing blunt ends. The protein Cas12a, which only has one component, recognises T-rich PAM at the target sequence's 5′ ends. With a 5′ overhang and staggered cuts, the DSB creates sticky ends. GE has also used the Cas12a (Cpf1) and Cas12b (C2c1) proteins from the class 2 type V CRISPR/Cas system in addition to the Cas9 proteins. The recognition of AT-rich PAM sequences by Cas12a and Cas12b as opposed to GC-rich PAM sequences by Cas9 is one of their many notable differences from Cas9 proteins (Khan *et al.*, 2021). Cpf1 requires a T-rich PAM sequence (5′-TTTN-3′ or 5′-TTTV-3′; V could be A, C, or G) near the conclusion of the protospacer region. In contrast to Cas9, which produces DNA breaks with blunt endpoints, Cpf1 produces DSBs with staggered ends at the distal position of a PAM, which may have additional advantages, particularly for knock-in approaches and may increase the effectiveness of the process of NHEJ-based gene insertion. Cas13, a newly discovered class II type VI CRISPR effector, may specifically target viral and endogenous RNAs in plant cells (Ahmad M., 2022; Ashraf *et al.*, 2022). Cas13

has been used to push proteins back to function in order to stop the disease's progression (Ahmad M., 2022; Ashraf *et al.*, 2022) Human cells have ADAR2 deaminase for RNA modification (converting adenosine to inosine. Its RNase activity is derived from the presence of higher eukaryotic and prokaryotic nucleotide-binding (HEPN) domain. Cytidine deaminase and dCas9 are used in the nuclear base editing system to choose the target site. Cytidine deaminase converts C directly into U, there is no double-strand break (DSB), and a CT substitution can be corrected during mismatch repair when the altered strand is used as a template. The REPAIR system uses dCas13 coupled to ADAR2 for RNA base editing, 50-nucleotide mRNA-gRNA duplexes, and 50-nucleotide RNA for "A-to-I" editing (Liu *et al.*, 2020; LaManna *et al.*, 2022; Li *et al.*, 2022; Liu G *et al.*, 2020; Liu Q *et al.*, 2020). Targeted base alterations (up to 40nt), insertions (up to 15 nt), and deletions can all be introduced with prime editing. Once inside, the fusion protein nicks the cell's DNA at the desired location, triggering the reverse transcription of the peg RNA's template sequence. The target cell is thereafter exposed to the peg RNA and fusion protein. The fusion protein nicks the target area of the target cell's DNA when the peg RNA and fusion protein are present in the target cell. This causes the DNA template present in the peg RNA to start being reverse-transcribed.

VI. FUTURE PROSPECTS

Our understanding of how the genome is structured and regulated in living cells from several biological kingdoms has already increased owing to CRISPR. Not only agriculture is being revolutionized by CRISPR, but also industry, the environment, medicine, and other professions. Due to the relative specificity of each nuclease platform, the majority of CRISPR/Cas9 genome-editing technology research has been preliminary up until this point. Future studies on the accuracy of each system's target recognition should take advantage of high-throughput methods that allow for detailed profiling of off-target cleavage sites. In order to be fully utilised, the platform must thus develop further, which will increase on-target efficacy. Several methods, such as modifying Cas9 to recognise different PAM sequences, have been used to get past this restriction. The xCas9 3.7 variant was created to boost the possibility of rice genome editing. In addition, SpCas9, a unique Cas9 variation, was created. The need to develop novel Cas9 variations that can recognise various PAMs is highlighted by the fact that many more of these variants are worthless in plants. The recently discovered Cas14a system, which does not require PAM but can only target ssDNA, can likewise be used to get past the restriction of PAM specificity. Therefore, the rapid growth of research into epigenetic genome modifications in rice is a viable technique for future rice crop improvement. As

CRISPR technologies expand in scope and power, social and ethical concerns about their use grow more pressing, and the potential applications of these powerful tools deserve greater thought. Researchers cannot disregard the challenges of communicating CRISPR breeding processes to the public in order to gain their trust and develop regulatory frameworks to control the deployment of the CRISPR system in agriculture. With the potential to ensure agriculture has a sustainable future, the recently developed CRISPR techniques are just the beginning. However, with that potential comes the responsibility to allay public and scientific concerns regarding the use of these potent new plant breeding technologies (Ahmad, 2023).

Questions Related To

1. Mechanism of CRISPR/ Cas system
2. Types of CRISPR/ Cas system
3. Advantages of CRISPR/ Cas over other genome editing tools
4. Current advances in CRISPR/ Cas system

Self Assesment

1. CRISPR/ Cas is an adaptive immunity system adopted from
   a. Bacteria
   b. Archaea
   c. Both a and b
   d. None of these

2. A distinct region used for the identification of target sequence in CRISPR/ Cas is
   a. Cas protein
   b. PAM
   c. Guide RNA
   d. None of these

3. A method requiring a homologous sequence to guide repair after gene editing in CRISPR system
   a. Non- homologous end joining
   b. Homology directed repair
   c. Both a and b
   d. DNA directed repair

4. Guide RNA has the following components
   a. crRNA
   b. tracr RNA
   c. Cas 9 enzyme
   d. Both a and b

5. PAM sequence of the SpCas 9 is
   a. 5'-NGG-3'
   b. 5'-NTT-3'
   c. 5'-NRR-3'
   d. 5'-TTAAA-3'

6. Knock out mutants are common in mechanism
   a. Homology directed repair
   b. Non- homologous end joining
   c. Both a and b
   d. Genome editing

7. Cas 12a was formerly known as
   a. C2c2
   b. Cas 13
   c. Cas 12a
   d. Cpf1

8. Cas 13a was formerly known as
   a. C2c2
   b. Cas 13
   c. Cas 12 a
   d. Cpf1

9. Find the odd one out
   a. ZFN - Zin fingers and Fok I
   b. TALEN - RVD and Fok I
   c. CRISPR - Guide RNA and Cas endonuclease
   d. CRISPR – RVD and Cas

10. T rich PAM sequences are the destination for the following genome editing tool
    a. Zinc finger nuclease
    b. CRISPR - Cas 12a system
    c. TALEN
    d. CRISPR -Cas 9 system

11. RNA targeted genome editing CRISPR/ Cas system
    a. Cas 9 system
    b. Cas 12a system
    c. Cas 13a system
    d. Cpf1 system'

12. Cas 9 system was found first in
    a. Streptococcus pyogenes
    b. Staphylococcus aureus
    c. Escherichia coli
    d. Salmonella typhi

13. CRISPR/ Cas system was first found in
    a. Streptococcus pyogenes
    b. Staphylococcus aureus
    c. Escherichia coli
    d. Salmonella typhi

14. PAM stands for
    a. Protospacer Adjunct Motif
    b. Protospacer Adjacent Motif
    c. Protoplasm Adjacent Mechanism
    d. Protoplacer Adjunct Mechanism

15. PAM sequences are identified by the following component of CRISPR/Cas system
    a. single guide RNA
    b. Cas 9 endonuclease
    c. Tracr RNA
    d. crRNA

16. The sequential order of the process of CRISPR/ Cas
    a. adaptation, expression/maturation and interference
    b. expression, interference and adaptation
    c. interference, expression and adaptation
    d. maturation, adaptation and maturation

17. Single guide RNA has a length of
    a. 5-7 bp
    b. 10-18 bp
    c. 18-22 bp
    d. 30-32 bp

18. First generation of genome editing tools include(s)
    a. Zinc Finger Nucleases
    b. TALENs
    c. CRISPR/ Cas system
    d. All of these

19. Cas 9 has the property of
    a. Nuclease
    b. Nickase
    c. Polymerase
    d. Both a and b

20. Cpf 1 stands for
    a. CRISPR Prevotella and Fransicella
    b. CRISPR Streptococcus
    c. CRISPR/ Cas 12 a
    d. None of these

21. Knock in mutants are produced by
    a. HDR
    b. NHEJ
    c. Site specific nucleases
    d. None of these

22. CRISPR stands for
    a. Clusters randomly Interlinked Short Palindromic Repeats
    b. Clustered Regularly Interlaced Small Protospacer Repeats
    c. Clustered Regularly Interspaced Short Palindromic Repeats
    d. Cas Regulated Interspaced Small Protospacer Repeats

23. Find the odd match
    a. Cr- CRISPR RNA
    b. tracr RNA – trans activating CRISPR RNA
    c. sg RNA- single guide RNA
    d. PAM – Palindromic Adjacent Motif Repeats

24. Cas protein is taken to the PAM sequence by the sgRNA, which has component(s) in Cas 12 a and component(s) in Cas 9
    a. One, One
    b. Two, two
    c. One, two
    d. Two, one

25. Type of cuts produced by the Cas 12a is and in Cas 9 is
    b. Blunt ends, Blunt ends
    c. Staggered ends, Staggered ends
    d. Blunt ends, Staggered ends
    e. Staggered ends, Blunt ends

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