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GENOME EDITING TOOLS

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Introduction:

Genome editing tools have the potential to change the genomic architecture of a genome at precise locations with the desired accuracy. These tools have been efficiently used for trait discovery in plants with high crop yields and resistance to biotic and abiotic stresses. There are several genome editing tools that have been developed to facilitate efficient genome editing, *viz.*, homologous recombination (HR), zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALENS), pentatricopeptide repeat proteins (PPRs), the CRISPR/cas9 system, RNA interference (RNAi), CIS genesis, and intragenesis. In addition, directed sequence editing and oligonucleotide-directed mutagenesis have the potential to edit the genome at the single nucleotide level. Recently, Adenine Base Editors (ABEs) have been developed to mutate A-T base pairs to G-C base pairs.

CRISPR-Cas 9 - CLUSTERED REGULARLY INTER SPACED SHORT PALINDROMIC REPEATS.

- CRISPR-Cas 9 is a technique that allows for the highly specific and rapid modification of DNA in a genome complete set of genetic instruction in an organism.
- It may favor the technique of editing because of its high degree of flexibility and accuracy in cutting and pasting DNA.
- One of the reasons for its popularity is the ability to conduct genetic engineering on an unprecedented scale at a very low cost.
- It differs from previous genetic engineering in that it allows for the introduction of more genes at a time.
- This technology is also being investigated for gene therapy, which aims to insert normal genes into the cells of people who suffer from genetic disorders such as cystic fibrosis, hemophilia, etc.
- A key breakthrough has been the development of a new cas9 fusion protein to act as a base editor.

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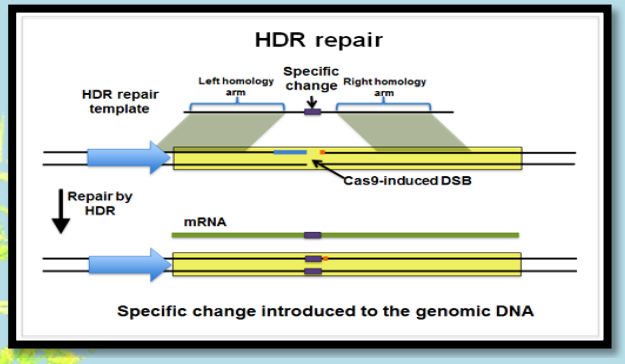
APPLICATIONS:

- The technology has been used to delete, insert, and modify DNA in human cells and other animal cells grown in petri dishes.
- Many scientists are using it to create transgenic animals such as mice, rats, pigs, and zebrafish.
- Use of CRISPR/cas9 in mice to eliminate muscular dystrophy, cure a rare liver disease, and make human cells immune to HIV
- The technology is also being investigated as a means to genetically engineer insects so as to wipe out insect-borne diseases such as malaria, transmitted by mosquitoes, and Lyme disease, transmitted by ticks.

HOMOLOGY DIRECTED REPAIR (HDR):

- Chromosomal recombination (HR) is the natural and most efficient genome engineering system that is present within the cell.
- Therefore, a similar mechanism with a minor or no error rate can be useful in genome editing technologies.
- This method can be used for genome editing through the initiation of double standard breaks (DSB) in the chromosome.
- DSB leads to meiotic recombination during cell division. DSBs are highly conserved in eukaryotes and can be initiated at specific sites, thus providing a great platform for gene targeting.

The frequency in somatic cells is very low, and hence the number of genomic modifications that are obtained by these techniques is very low.



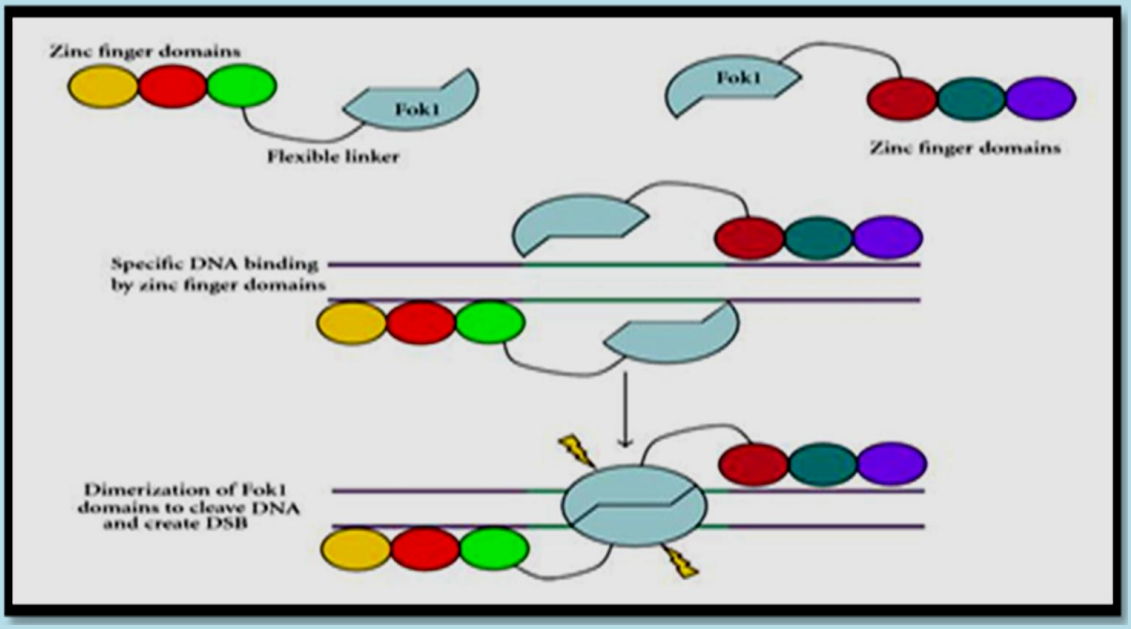
ZINC FINGER NUCLEASE:

- Zinc finger nucleases are artificial restriction enzymes generated by fusing a zinc finger DNA-binding domain with a DNA-cleavage domain.
- Zinc finger domains can be engineered to target specific desired DNA sequences, and this enables zinc finger nucleases to target unique sequences within complex genomes.
- By taking advantage of endogenous DNA repair machinery, reagents are used to alter the genomes of higher organisms.

- ZFN is a prominent tool in the field of genetic engineering.
- ZFN are targetable DNA cleavage reagents that have been adopted as gene-targeting tools.
- ZFN-induced double-strand breaks are subject to cellular DNA repair processes that lead to both targeted mutagenesis and targeted gene replacement at remarkably high frequencies.
- Zinc finger nuclease technology heralds a new era in mammalian transgenesis.

TRANSCRIPTION ACTIVATOR LIKE EFFCTOR NUCLEASE (TALENs):

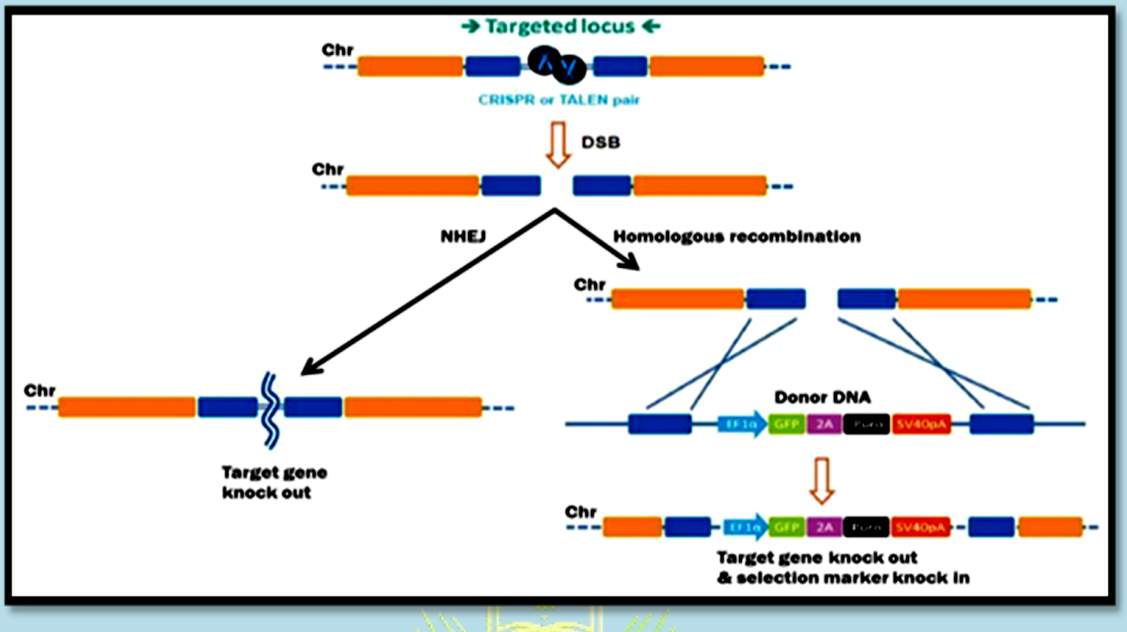
- TALEN are restriction enzymes that can be engineered to cut specific sequences of DNA.
- They are made by fusing a TAL effector DNA-binding domain with a DNA-cleaving domain.



TARGET APPLICATONS:

- Functional Genomics.
- Cell-based screening
- Cell line optimization
- Development of large animals' models for human disease and xenotransplantation
- Agricultural breeding.
- Transcription activator-like effectors can be engineered to bind to practically any desired DNA sequence, so when combined with a nuclease, DNA can be cut at specific locations.
- TALEN is a prominent tool in the field of genome editing.

- The site-specific chromosomal double-strand breaks introduced by TALENs significantly increase the efficiency of genomic modification.
- Recent computational and structural studies have provided new insights into how they recognize RNA, and the recognition is sequence-specific.



APPLICATION OF TALENs:

- Outside of plants and algae, TALENs have been used successfully to modify genes in yeast, fruit fly, roundworm, crickets, zebrafish, frog, rat pig, cow, silkworm, and humans.
- TALEN-based editors were used to successfully edit the mitochondrial DNA within living cells in a precise manner.

PENTATRICOPEPTIDE REPEAT PROTEIN(PPRs):

- Pentatricopeptide repeat proteins control RNA metabolism across the eukaryetic domain.
- The modular code for RNA building by PPR proteins holds great promise for the engineering of new tools to target RNA and identify RNAs around natural PPR proteins.
- A typical PPR protein is targeted at mitochondria or chloroplasts, binds one or several organellar transcripts, and influences their expression by altering RNA sequences, processing, or translation.
- Their combined action has prefound effects on organelle biogenesis and function and consequently on

photosynthesis, respiration, and environmental responses.

- It provides a pathway towards designing synthetic RNA-binding proteins aimed at a desired target.

CONCLUSION

ZFN and DSBs can potentially be used for precise genome editing in plants and have a huge impact on functional genomics studies. Targeted mutation-related breeding methods can use precise genome editing at specific sites rather than random mutations. Genome editing technology has great potential for revolutionizing crop production worldwide. The CRISPR/cas 9 system can be very useful post transcriptional control of gene expression and it will be very useful for generating point mutations. From the database, suitable genome editing tools for complex genes could be found by a researcher. The introduction of a "genome editing" database with harder experimental references as well as in-silico prediction data for model organisms could be of particular interest.

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