Article Title (3 to 12 words)	**CRISPR**: A New Era Genome Editing Tool
Article Summary (In short - What is your article about – Just 2 or 3 lines)	CRISPR is the latest genome editing tool developed by clubbing the strategies from the age old known bacterial immune system and the homology directed DNA repair mechanism which lets us introduce desirable mutations precisely at known sites of the host genome and is better than the other players of genome editing niche namely ZnF and TALEN.
Category	**Genetics**
Your full article (between 500 to 5000 words) - -	Do check for grammatical errors or spelling mistakes

CRISPR: A New Era Genome Editing Tool

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What is CRISPR?

CRISPR is a defense mechanism found naturally in the bacteria to guard it against the attack of bacteriophages or other viral particles. It was first discovered by Francisco Mojico in 1993 in

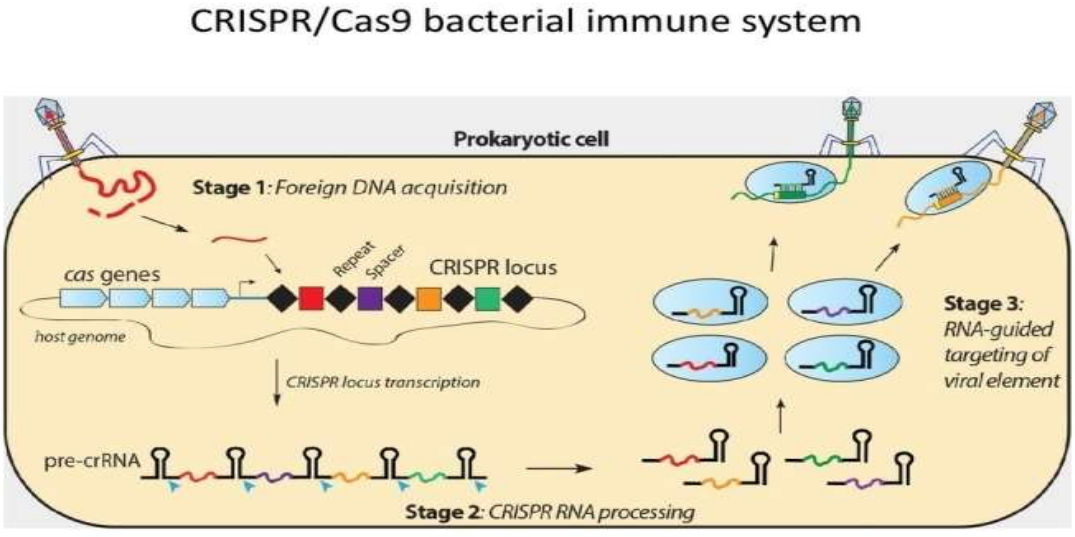

Figure 1.The figure shows the CRISPR associated defense mechanism in bacterial cells

Streptococcus pyrogenes which causes strep throat.

CRISPR refers to '**C**lustered **R**egularly **I**nter-spaced **S**hort **P**alindromic **R**epeats' which refers to the black rhombus (Fig.1) that is regularly spaced and the coloured rectangles spanned in between the black rhombus are called Spacer regions which get in between the repeats when a bacteriophage attacks but its DNA is ruptured and one of its fragment is captured in the spacer region. Each coloured rectangle represents a dsDNA fragment belonging to a particular bacteriophage. In the figure given above (Fig.1) we can see that there are dsDNA fragments picked up from four different bacteriophages (Shown in four different colours: **Red**, **violet**, **amber** and **green**). The sequencing results of these spacer regions show that all of them share a common sequence at one end called the **PAM** (**P**roto-spacer **A**djacent **M**otif).The repeats (**black** rhombus in figure 1.) remain adjacent to the spacer regions and are

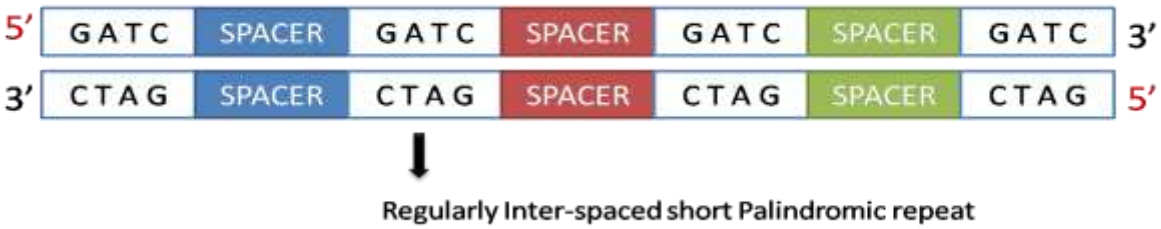

Figure 2. Schematic representation of the repeats and spacers in CRISPR locus

Palindromic in nature. The Palindromic nature of the repeats is shown more clearly in figure 2.

The whole cassette of the CRISPR locus is transcribed as a single unit under a common promoter and is called pre-CRISPR RNA which is cleaved to give rise to individual CRISPR RNAs specific to individual bacteriophages. Now each CrRNA (CRISPR RNA) gets loaded in to individual Cas9 endonucleases. The Cas9 (CRISPR Associated Protein 9) is an endonuclease that binds to the structural conserved domain of the CRISPR RNA leaving the region transcribed from the spacer region open for binding to its corresponding complementary sequence present in the target host bacteriophage genome. After the Riobo-nucleo-protein complex is bound to the target host bacteriophage genome the host genome gets cleaved at the site of complementarity usually just 3 bp upstream of the PAM site. Thus the bacterium stays unaffected (See Fig.1).

Now talking exclusively about the Cas9 endonuclease, it has been found that the endonuclease is quiet target specific when it cleaves. It was first discovered by Alexander Bolotin in 2005 in *Streptococcus thermophilus*. Taking the advantage of its site specific cleavage activity scientists have now found a way to introduce site specific mutations anywhere in the genome to correct gene defects or to prematurely stop the translation of undesirable proteins (in a particular pathway by introducing mutations at the promoter region or in the coding region) to deviate the pathway to maximize out the desirable product of interest by clubbing this capability of the Cas9 endonuclease with the well known Homology-directed DNA repair mechanism.

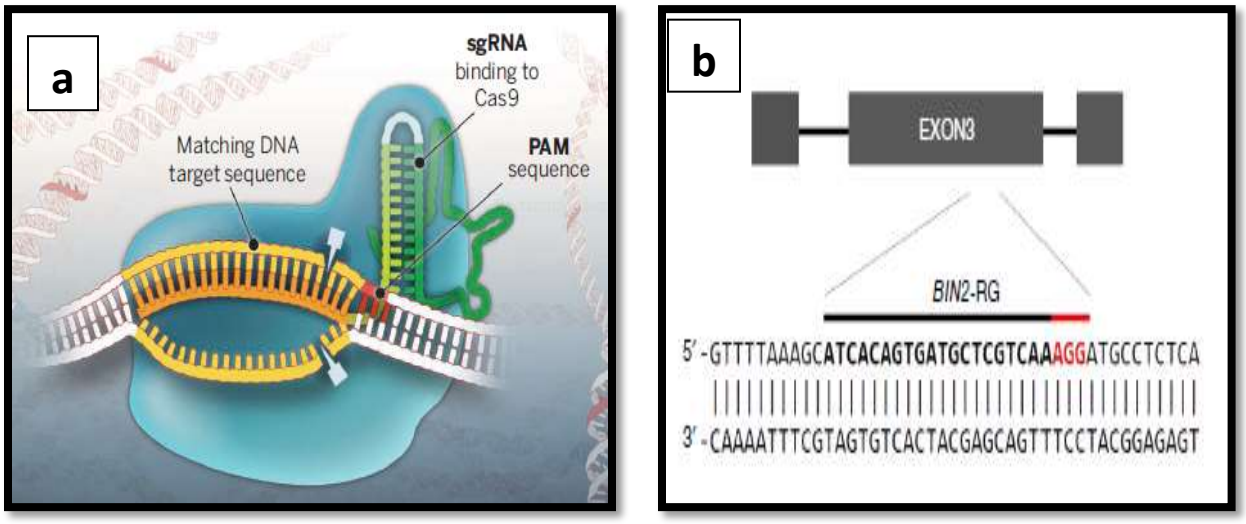

Figure 3. a) Shows the CrRNA(here sgRNA)in the ribo-nucleo-protein complex binding just adjacent to the downstream of the PAM sequence. The site of cleavage is mostly 3 bp downstream of the PAM sequence. The PAM site is always present in the target host site and not designed in to the sgRNA. The host complementary region designed in an sgRNA should just precede the PAM site in the target genome and should be maximum of 20-22 bp for better target specificity. b) Shows the target region of a gene called *BIN2* (Brassinosteroid Insensitive 2 which negatively regulates Brassinosteroid signaling pathway) present in lettuce for designing sgRNA to inactivate the gene by introducing specific mutation at the given particular site. The PAM site is always of 3bp and has a sequence of '**NGG**' where N can be any nucleotide. The PAM site in the figure is shown in red and the complementary region to design the sgRNA is shown in **black** block letters in the host target region.

Now the next thing is how exactly CRISPR/Cas9 and the homology directed DNA repair mechanism play together to get our desired mutation. The fact is just after the cleavage; Cas9 leaves blunt DNA ends which trigger the natural DNA repair mechanism of the cell. These are of two types: Non-homologous end joining (NHEJ) and Homology-directed repair mechanism. A brief schematic overview of both the repair mechanism types is shown in the figure 4.

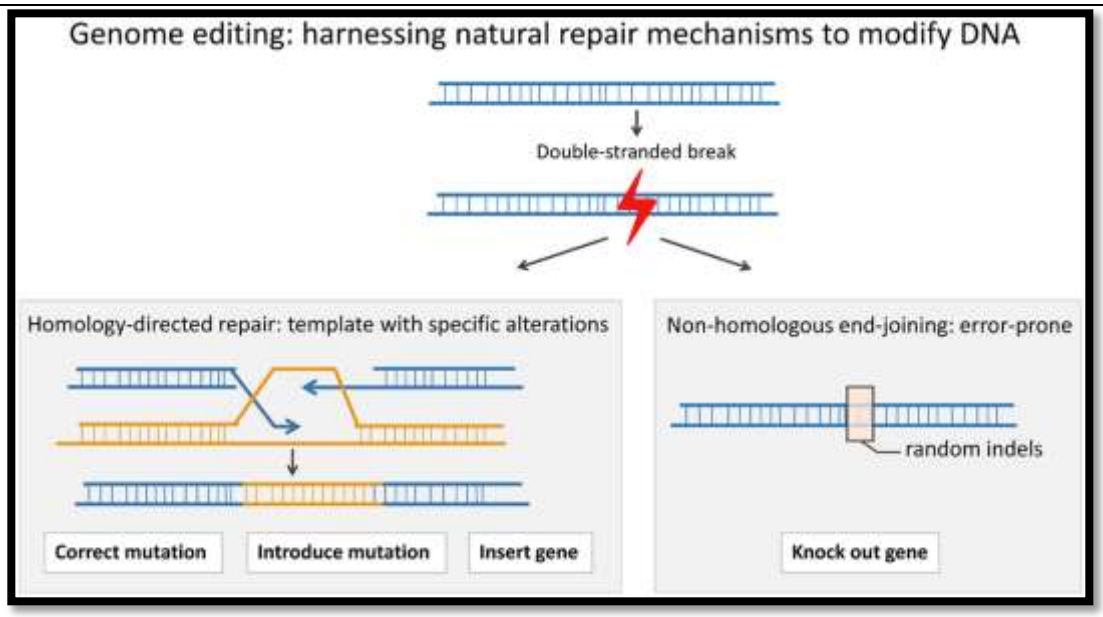

Figure 4. The figure shows the fate of a DNA after getting a DSB (Double-stranded break). The first possibility (shown in the left side) is if there is any homologous sequence found in the adjacent chromosome trimming at the 5' end will take place to generate staggered end followed by strand invasion from the broken DNA regions in to the homologous sequence found in the adjacent chromosome. The respective complementary strands of the adjacent homologous sequence will be used as template to elongate the lost region by DNA Pol I (or II) and then they will come back to their original position and the nicks will be sealed by ligase. Note that if there is any mutation in between the points just from where strand invasions start the mutations can be taken up without any notice. The second possibility (shown in the right side) is Ku70/Ku80 heterodimer will bind to the blunt DNA ends which will engage another protein called Artemis which will cleave the DNA ends which is the main region of getting mutations. The final ligation will be done by DNA ligase IV engaged by XRCC4. During this repair insertion may also occur.

Until now, as we have understood the basics of both CRISPR/Cas9 and HDR (Homology-directed DNA repair) from the above given information, it's now time to figure out the real deal. Commercial CRISPR/Cas9 systems available in the markets are binary vector systems. Each set has two vectors: First the CRISPR/Cas9 vector and second the homology vector (also called the donor vector) containing the desired mutation in between the LHR (Left Homologous Region) and the RHR (Right Homologous Region). Whereas the systems those need to introduce a non-specific (any indel) mutation at a specific host site don't require the homology vector. In the

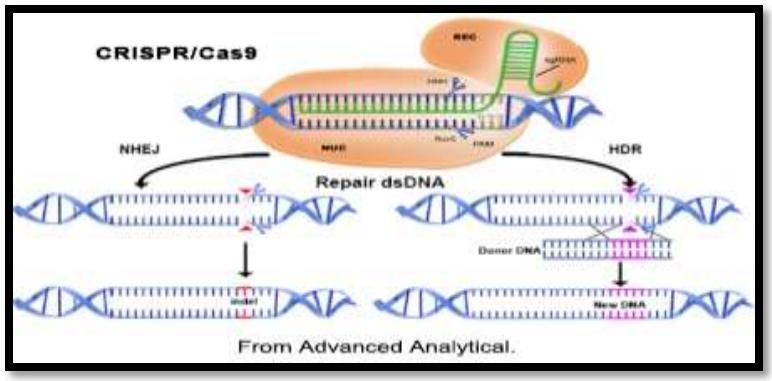

Figure 5. Shows how a donor DNA vector can be used for inducing HDR in CRISPR/Cas9 genome editing system

former case when we need to introduce a patch of mutation (5-10 or more bp substitution) the approach is the new patch of sequence has to be put in between the exact homologous sequence right after and before the patch of mutation flanking both the sides as LHR and RHR. Crossing over will take place at the LHR and RHR (after the Cas9 cut is made and the homology vector will approach the cut site as a source of HDR mechanism) which will swap the correct sequence in the host target site with the mutated once other flanking things remaining unchanged. This approach can also be used for introducing additions.

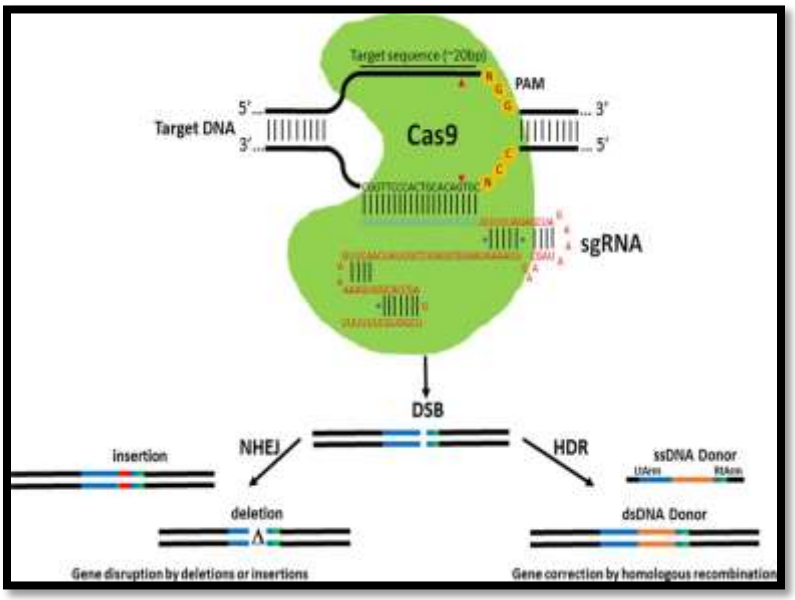

Figure 6. This figure shows a very important note point i.e. ssDNA Donor can also be used to induce HDR in CRISPR/Cas9 genome editing system

Genome Editing tools other than CRISPR/Cas9:

1. Zinc finger proteins (ZNFs) were the first of the "genome editing" nucleases to hit the scene. Zinc fingers are the most common DNA binding domain found in eukaryotes. They typically are comprised of ~ 30 amino acid modules that interact with nucleotide triplets. ZNFs have been designed that recognize all of the 64 possible trinucleotide combinations, and by stringing different zinc finger moieties, one can create ZNFs that specifically recognize any specific sequence of DNA triplets. Each ZNF typically recognizes 3-6 nucleotide triplets. Because the nucleases to which they are attached only function as dimers, pairs of ZNFs are required to target any specific locus: one that recognizes the sequence upstream and the other that recognizes the sequence downstream of the site to be modified.

2. TALENs – Transcription activator-like effector nucleases - are similar to ZNFs in that they use DNA binding motifs to direct the same non-specific nuclease to cleave the genome at a specific site, but instead of recognizing DNA triplets, each domain recognizes a single nucleotide. The interactions between the TALEN-derived DNA binding domains and their target nucleotides are less complex than

those between ZNFs and their target trinucleotides, and designing TALENs is generally more straightforward than ZNFs.

Advantages of CRISPR/Cas9 over ZnF and TALEN

1. **Target design simplicity**. Because the target specificity relies on ribonucleotide complex formation and not protein/DNA recognition, gRNAs can be designed readily and cheaply to target nearly any sequence in the genome specifically.

2. Efficiency. The system is super-efficient. Modifications can be introduced by directly injecting RNAs encoding the Cas protein and gRNA into developing mouse embryos. This eliminates the long and laborious processes of transfecting and selecting mouse ES cells that are required to create targeted mutant mice using classical homologous recombination techniques.

3. Multiplexed mutations. Mutations can be introduced in multiple genes at the same time by injecting them with multiple gRNAs.

Examples of successful use of CRISPR/Cas9 system in Genome Editing

Organism	Cell line	Targeted gene	Repair pathway	Efficiency, %
Homo sapiens	H293K	AAVS1	HR/NHEJ	10–25
Homo sapiens	K562	AAVS1	NHEJ	8–13
Homo sapiens	PGP1 iPS	AAVS1	HR/NHEJ	2–4
Homo sapiens	H293FT	EMX1, PPVALB, EMX1	HR/NHEJ	Up to 21
Mus musculus	Neuro2A	Th	NHEJ	Up to 27
Danio rerio	Embryo	etsrp, gata4, or gata5	HR/NHEJ	Up to 25
Mus musculus	Embryo	Tet1, Tet2, Tet3, Sry, Uty	HR/NHEJ	Up to 48
Saccharomyces cerevisiae	BY4733	CAN1	HR/NHEJ	Near 100
Caenorhabditis elegans	Embryo	*unc-119, dpy-13, klp-12a, Y61A9LA.1a*	NHEJ	Up to 80
Drosophila melanogaster	Embryo	*y1, y2, w1, w2*	NHEJ	Up to 88
Arabidopsis thaliana	–	*AtPDS3, AtFLS2, AtRACK1b*, and *AtRACK1c*	NHEJ	Up to 5.6
Nicotiana benthamiana	–	*NbPDS3*	HR/NHEJ	Up to 38.5
Rice (*Oryza sativa*)	–	*OsPDS, OsBADH2, Os02g23823, OsMPK2*	HR/NHEJ	Up to 38
Common wheat (*Triticum aestivum*)	-	*TaMLO*	NHEJ	28.5
Bacteria	*Streptococcus pneumoniae* and *Escherichia coli*	*bgaA, srtA, ermAM, rpsL*	HR/NHEJ	Up to 100

Gasiunas *et al.,* 2015

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