Genome Editing

Genome Editing (also called **gene editing**) is a group of technologies that give scientists the ability to change an organism's DNA. These technologies allow genetic material to be added, removed, or altered at particular locations in the genome, resulting in inactivation of target genes, acquisition of novel genetic traits and correction of pathogenic gene mutations.

Applications of genome editing:

In recent years, with the rapid development of life sciences, genome editing technology has become the most efficient method to [1]study gene function, explore the pathogenesis of hereditary diseases, [2]develop novel targets for gene therapy, [3]breed crop varieties and more.

Principles of genome editing:

During genome editing process, a piece of DNA is inserted, replaced, or removed from the organism's genome, with the help of genetically engineered nucleases (that were engineered to recognize specific target sequences) and the host cell's own double-stranded break repair machinery. Endonuclease binds to a specific site in the genome to generate a double-stranded break (**DSB**), which can be repaired by two endogenous self-repair mechanisms, the error-prone non-homologous end joining (**NHEJ**) pathway or the homology-directed repair (**HDR**) pathway . Under most conditions, NHEJ is more efficient than HDR, for it is active in about **90%** of the cell cycle and not dependent on nearby homology donor . NHEJ can introduce random insertions or deletions into the cleavage sites, leading to the generation of frameshift mutations or premature stop codons within the open reading frame (ORF) of the target genes, finally inactivating the target genes . Alternatively, HDR can introduce precise genomic modifications at the target site by using a homologous DNA repair template (**Fig. 1**).

There are currently four families of engineered nucleases used for genome editing purposes:

1. Zinc finger nucleases (ZFNs)
2. Transcription activator-like effector nucleases (TALENs)
3. Engineered meganucleases
4. The CRISPR/Cas9 system

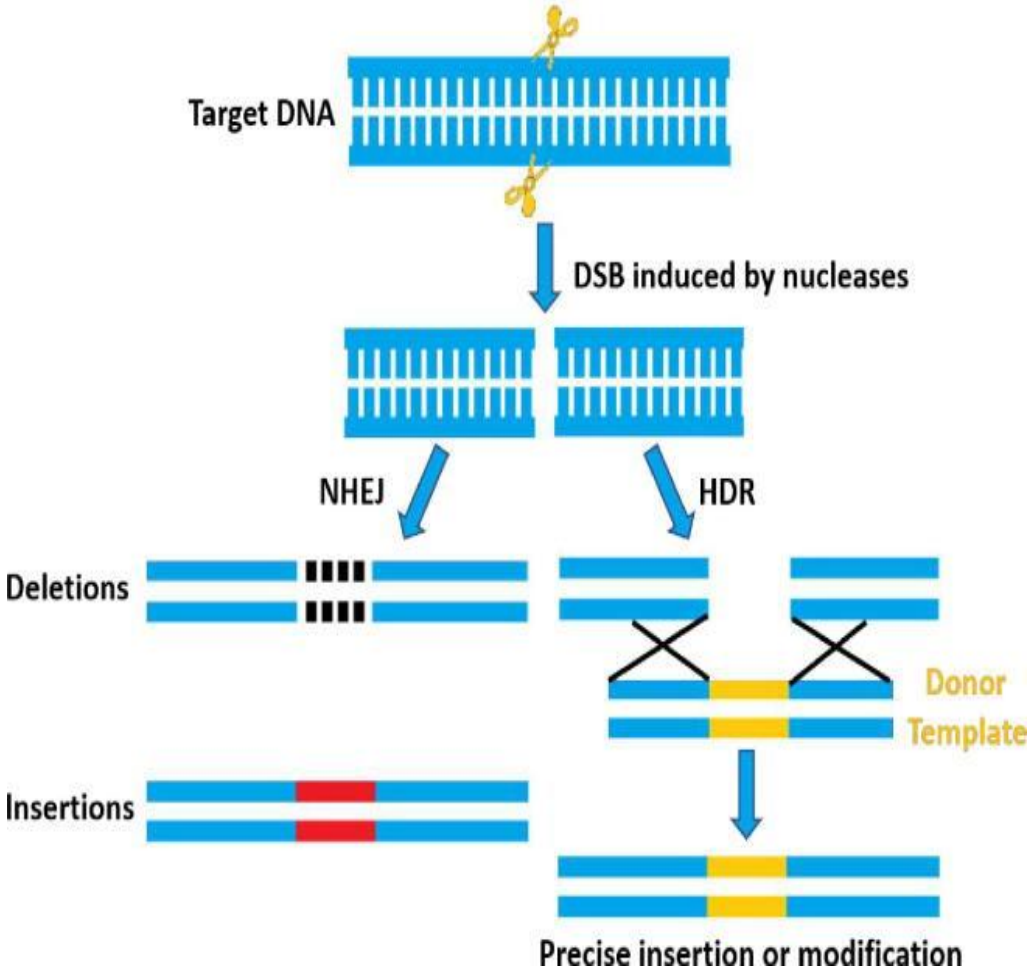


Fig.1: Mechanism of genome editing. Double-strand break (DSB) induced by nucleases can be repaired by non-homologous end joining (NHEJ) or homology-directed repair (HDR) pathways. NHEJ can introduce random insertions or deletions (indels) of varying length at the site of the DSB. Alternatively, HDR can introduce precise genomic modifications at the target site by using a homologous DNA donor template.

1-Zinc finger nucleases (ZFNs): are a class of engineered DNA-binding proteins that facilitate targeted editing of the genome by creating double-strand breaks in DNA at user-specified locations. are based most typically on the **Fok I** restriction enzyme that is fused to a zinc finger DNA-binding domain engineered to target a specific DNA sequence.

2-Transcription activator-like effector nucleases (TALENs) : Are **e**ngineered nucleases enable the targeted alteration of nearly any gene in a wide range of cell types and organisms. The **TALENs** are similar to ZFNs. Each DNA-binding domain of TALENs can recognize a different single DNA base, hence a combination of different TALENs can in practice be used to target any

specific sequence on the genome. The endonuclease activity again is through the **Fok I** restriction enzyme. **TALENs** have major advantages over **ZFNs**. First, off-target mutation rates are generally lower, and they can be designed to target virtually any genomic sequence.

3-Engineered meganucleases:

Are endodeoxyribonucleases of microorganisms, act as molecular DNA scissors characterized by a large unique recognition site (double-stranded DNA sequences of **12** to **40** base pairs); this site generally occurs only once in any given genome. *For example*, the **18-base** pair sequence recognized by the **I-SceI** meganuclease would on average require a genome twenty times the size of the human genome to be found once by chance (although sequences with a single mismatch occur about three times per human-sized genome). Meganucleases are therefore considered to be the most specific naturally occurring restriction enzymes**.**

Meganucleases have been known to cause less toxicity in cells compared to ZFNs or TALENs.

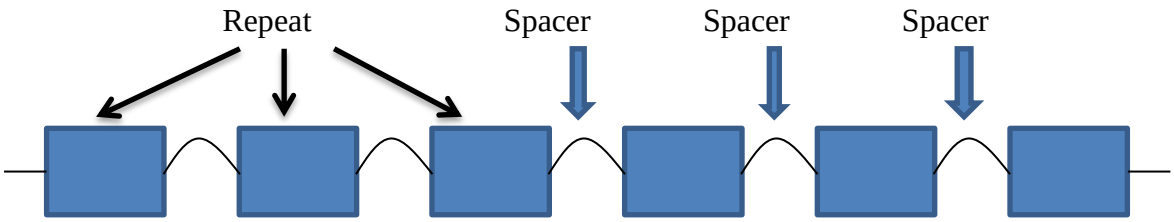
Meganucleases are used to modify all genome types, whether bacterial, plant or animal. They open up wide avenues for innovation, particularly in the field of human health, for example the elimination of viral genetic material or the "repair" of damaged genes using gene therapy.

4- The CRISPR/Cas9 system:

CRISPR: is clustered, regularly interspaced, short palindromic repeat and the associated Cas9 protein. CRISPRs are found in approximately **40%** of sequenced bacterial genomes and **90%** of sequenced archaea.

Cas 9 is an endonuclease, first identified from ***Streptococcus pyogenes*** bacteria. It's genes are often located next to CRISPR repeat-spacer arrays.

CRISPR-Cas9 was adapted from a naturally occurring genome editing system that bacteria use as an immune defense. When infected with viruses, bacteria capture small pieces of the viruses' DNA and insert them into their own DNA in a particular pattern to create segments known as CRISPR arrays(viral DNA fragments ("spacers" **17-84** bases long), separated by short palindromic repeats (**23-50** bases) and grouped into clusters in intergenic regions.



The CRISPR arrays allow the bacteria to "remember" the viruses (or closely related ones). If the viruses attack again, the bacteria produce RNA segments from the CRISPR arrays that recognize and attach to specific regions of the viruses' DNA. The bacteria then use Cas9 or a similar enzyme to cut the DNA apart, which disables the virus (**Fig.2**).

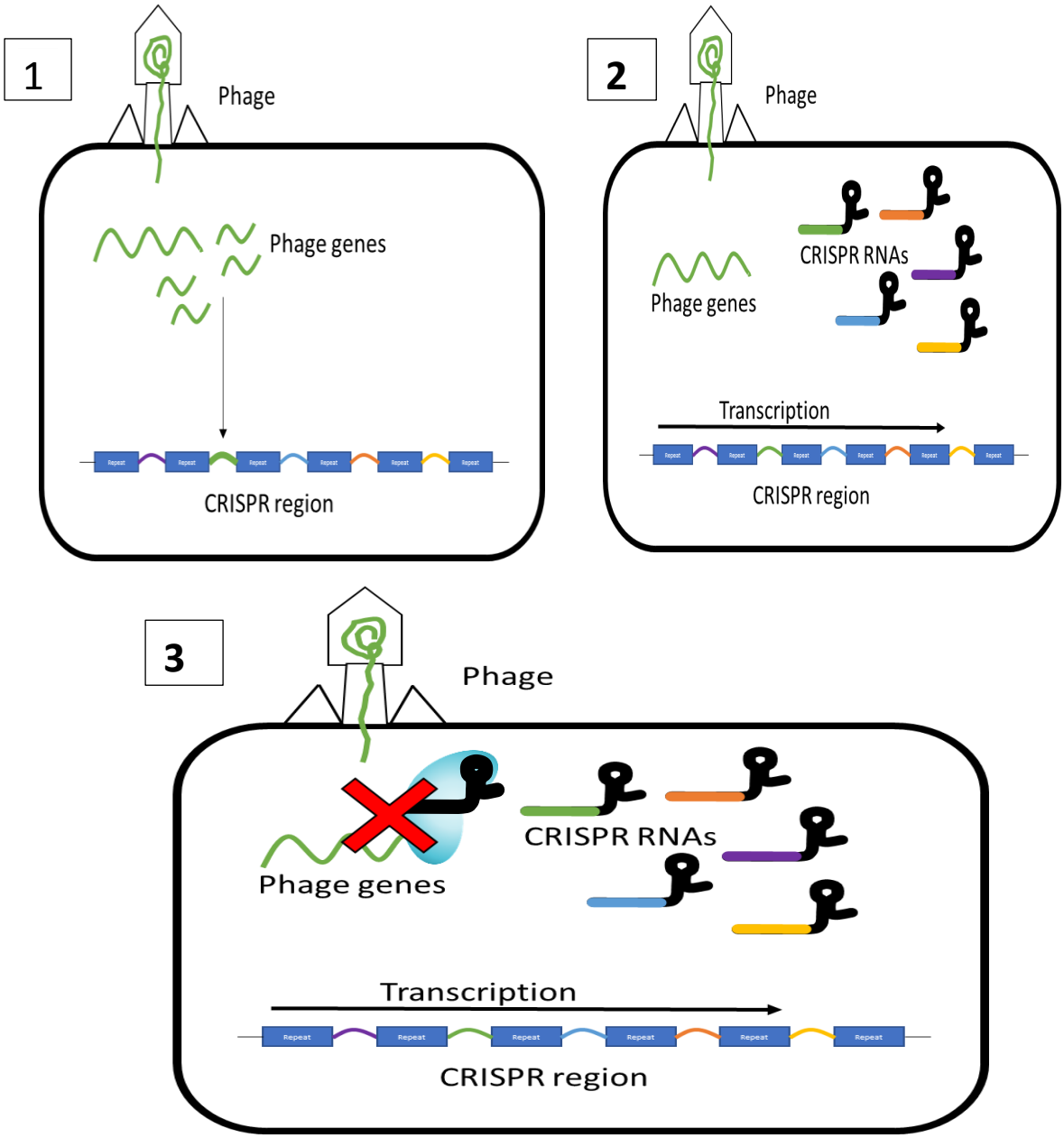


Fig.2: Natural genome editing system in bacteria**.**

Specificity of CRISPR-Cas9 depends on the presence of a sequence- specific **Protospacer Adjacent Motif** (**PAM**) and target sequence (**20 bases**). Absence of PAM in host genome enable to avoid self-cleavage. Remember the job of Cas9 is to generate **DSB** in target sequence and incorporate and store it as memory to combat future bacteriophage invasion. And that's why it called an Adaptive immunity system(**Fig 3**).

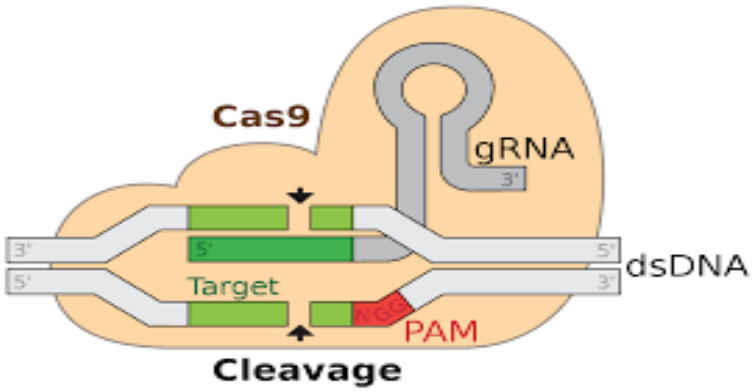


Fig 3: Combination of gRNA and CAS9 near the PAM to cleavage the target DNA.

CRISPR-Cas systems have become the most favorite genome editing tool in the molecular biology laboratory all around the world since they were confirmed to have genome editing capabilities in 2012**,** due to the advantages of simple design, low cost, high efficiency, good repeatability and short-cycle.

Researchers adapted this immune defense system to edit DNA. They create a small piece of RNA with a short "guide" sequence that attaches (binds) to a specific target sequence in a cell's DNA, much like the RNA segments bacteria produce from the CRISPR array. This guide RNA also attaches to the Cas9 enzyme. When introduced into cells, the guide RNA recognizes the intended DNA sequence, and the Cas9 enzyme cuts the DNA at the targeted location, mirroring the process in bacteria. Although Cas9 is the enzyme that is used most often, other enzymes (for example **Cpf1**) can also be used. Once the DNA is cut, researchers use the cell's own DNA repair machinery to add or delete pieces of genetic material, or to make changes to the DNA by replacing an existing segment with a customized DNA sequence.

Genome editing is of great interest in the prevention and treatment of human diseases. Currently, genome editing is used in cells and animal models in research labs to understand diseases. Scientists are still working to determine

whether this approach is safe and effective for use in people. It is being explored in research and clinical trials for a wide variety of diseases, including single-gene disorders such as cystic fibrosis, hemophilia, and sickle cell disease. It also holds promise for the treatment and prevention of more complex diseases, such as **cancer**, **heart disease**, **mental illness**, and **human immunodeficiency virus** (**HIV**) infection.

Ethical concerns arise when genome editing, using technologies such as CRISPR-Cas9, is used to alter human genomes. Most of the changes introduced with genome editing are limited to somatic cells, which are cells other than egg and sperm cells. These changes are isolated to only certain tissues and are not passed from one generation to the next. However, changes made to genes in egg or sperm cells or to the genes of an embryo could be passed to future generations. Embryo genome editing bring up a number of ethical challenges, including whether it would be permissible to use this technology to enhance normal human traits (such as **height** or **intelligence**). Based on concerns about ethics and safety, embryo genome editing are currently illegal in the United States and many other countries.