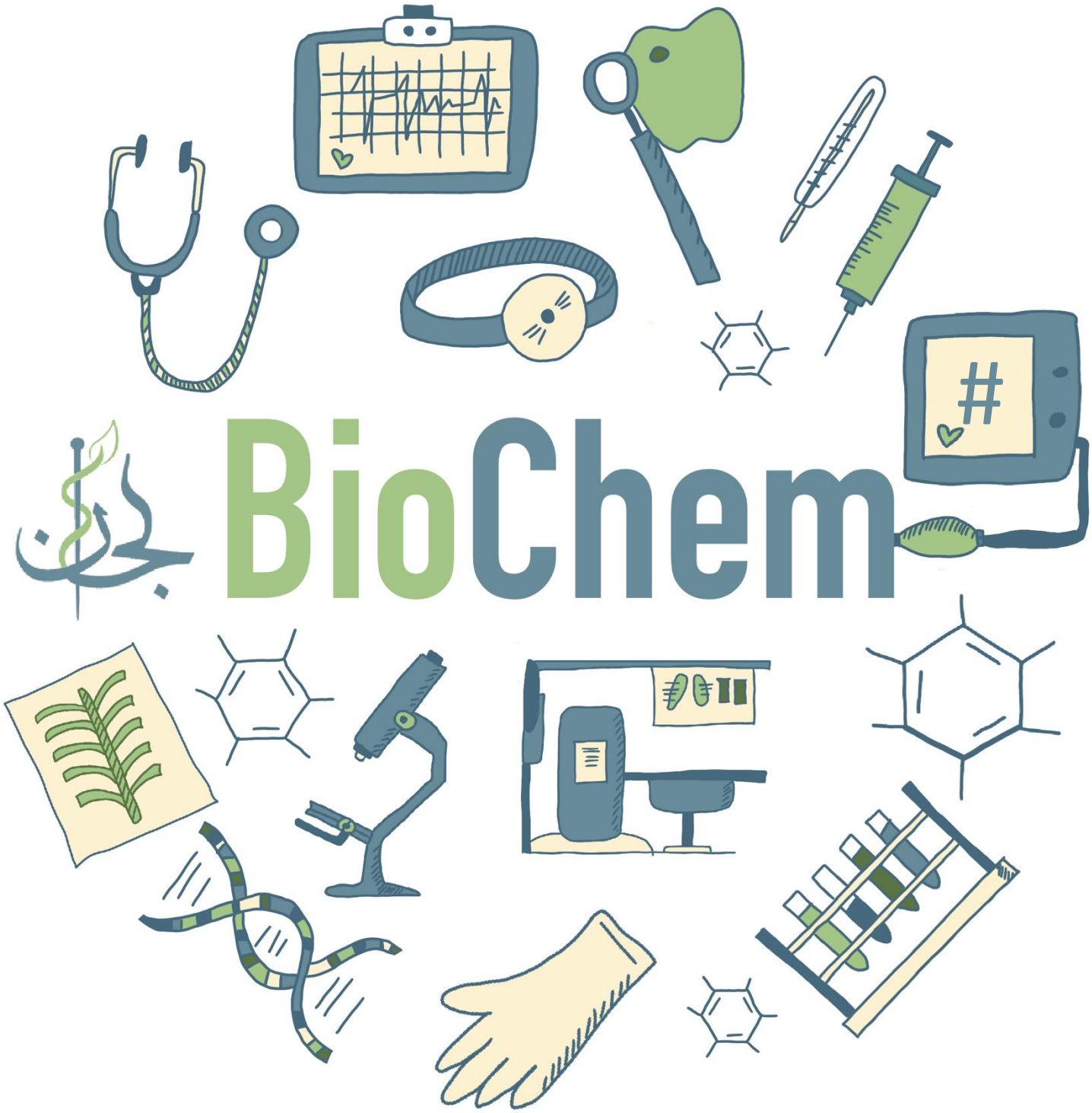

BioChem

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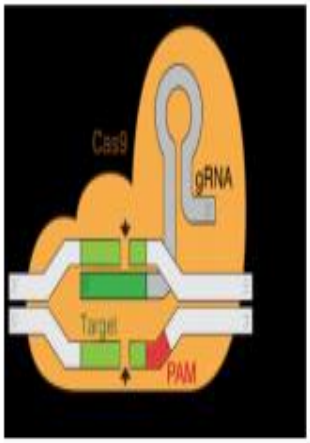
In this sheet we are going to talk about a new technology known as " CRISPR /Cas9" system that is frequently used in gene editing .

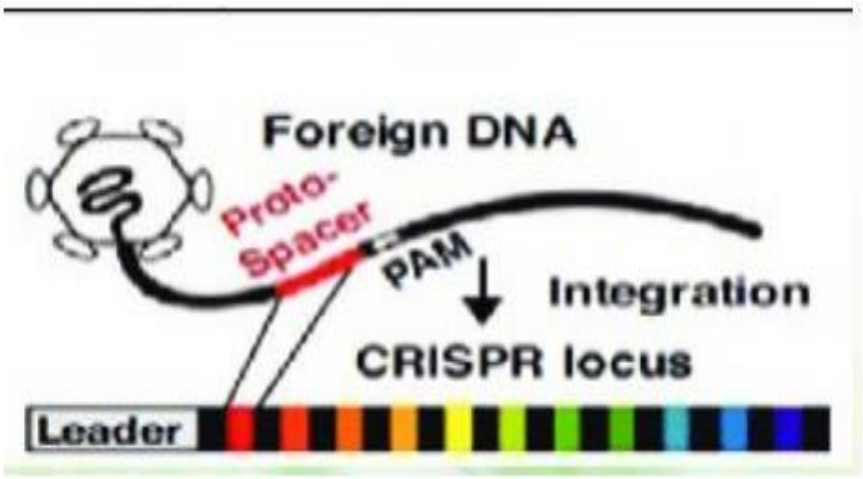
CRISPR-CAS9 system

CRISPR is an abbreviation for Clustered regularly interspersed short Palindromic repeats . It is a bacterial genetic system that constitutes the immune system of bacteria against phages.

Cas9 is **nuclease** (Enzyme that degrades nucleic acids) and it is RNA-guided that can either create single or double strand breaks, so it is associated with a short single-stranded RNA molecule known as **guide RNA (g-RNA**) or **single guide RNA (sg-RNA**) and this RNA molecule guides the nuclease to a certain sequence within the structure of the DNA that is COMPLEMENTARY to the RNA sequence. So it is a **ribonucleoprotein**

Recall that a **Ribonucleoprotein** is composed of a protein part (the Nuclease) and an RNA nucleic acid part .





- In the previous image ,The black regions are the palindromic repeats and the colored are portions of the bacteriophage DNA that gets inserted into the bacterial chromosome. the palindromic repeats as you see are interspaced (they are separated from each), regularly organized (that is , separated by the same distance) and clustered in one area in bacterial DNA (bacterial chromosome) .

- The Main purpose of the CRISPR-CAS9 System in bacterial cells is to protect bacterial cells from **Bacteriophages** (Viruses that invade and cause infections in bacterial cells) .

-The Mechanism of bacteriophages:-

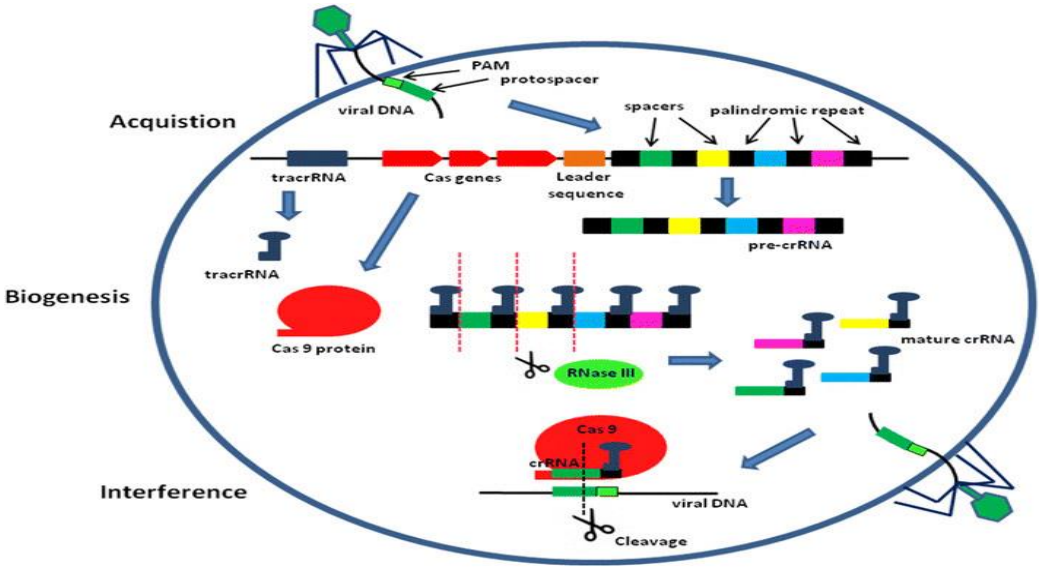
1- Bacteriophages sit on the plasma membrane of bacterial cells and infect them by inserting their DNA and they take over the whole system by utilizing the transcriptional machinery of the host bacterium.

2- Then, they multiply inside cells and cells eventually rupture, releasing a lot of bacteriophages that would travel and infect neighboring cells .

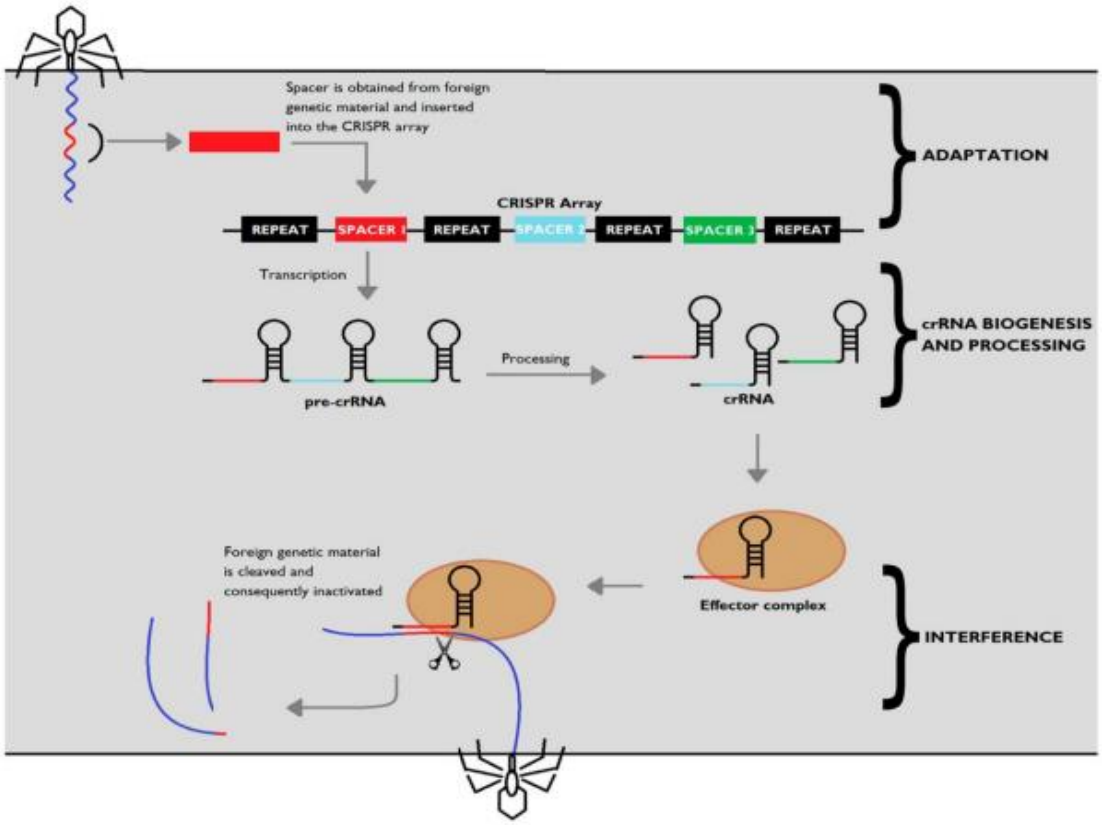
-**How Can bacterial cells prevent this:-**

1- In order for Bacterial cells to protect themselves , when a phage infects a bacterial cell ,they are able to degrade (chop off) the Bacteriophage DNA into smaller pieces by perhaps utilizing **a restriction endonuclease** and integrate one of these fragments into the CRISPR cluster .

2- When the phage infects the cell again , the cell transcribes the DNA into an RNA that undergoes processing as shown in the next image (guide RNA or gRNA) , which is then integrated in the CAS-9 Nuclease thereby guiding the nuclease to the phage DNA to degrade it . (Keep in mind that the guide-RNA have this ability to guide because it is COMPLEMENTARY to the Phage DNA) .



Note : The palindromic repeats in the CRISPR sequence are transcribed and they aid in fixing the gRNA to the CAS9 Nuclease

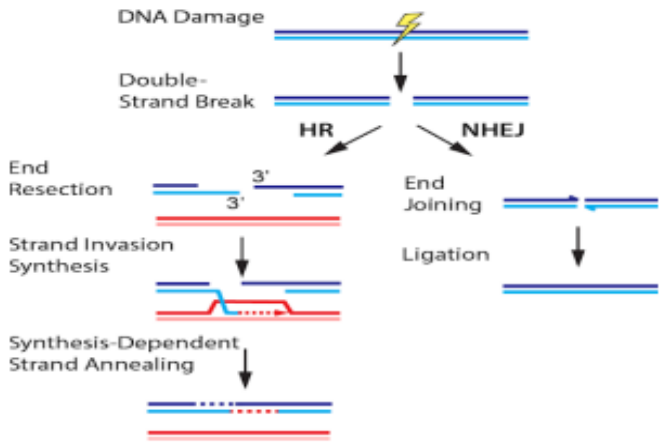


black repeats are the palindromic, red is a part acquired of the bacteriophage DNA, blue is actually a bacteriophage that infected bacterial cells previously.

In the previous image, the Bacterial cell took part of the degraded newly-entered viral DNA (Phage-DNA) and inserted it into its own chromosome Between the Palindromic repeats . The same thing with the green DNA and so on. What happens is that you have another bacteriophage (the one at the bottom, the same as the one above) trying to infect the bacterial cell . BUT this bacteriophage is not successful in infecting cells. The reason is that the **CRISPR genetic system** is transcribed into RNA and this RNA associates with Cas9 enzyme. thereby guiding the Cas9 to the bacteriophage DNA and degrading it . Don't forget that RNA here is complementary to the bacteriophage DNA so it associates to it and Cas9 degrades and cleaves the bacteriophage DNA. So that it can't infect the bacterial cell .

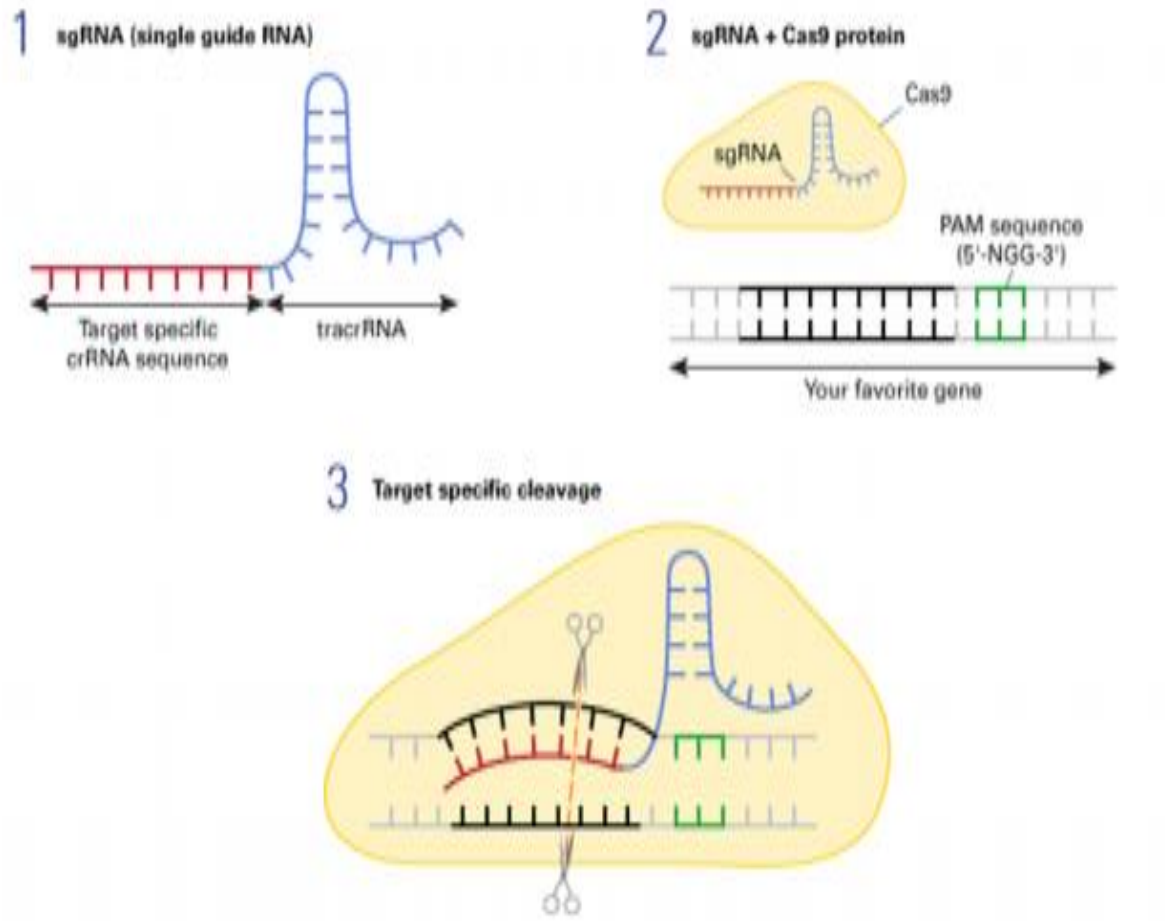
Note: We can think of the incorporated Phage-DNA fragments (Also known **as Spacers)** as the memory of the bacterial cells representing indicating that the a bacteriophage had previously tried to infect this bacterial cell and in doing so , the bacterial cell degraded its DNA while in the same time incorporating these spacers within its genome .

DNA repair mechanisms in human cells



- When DNA is damaged (Double stranded breaks) , two recombinant repair mechanisms are activated :-

The first one is known as " homologous recombination-repair (HR)	**The second repair mechanism is " Non- homologous End Joining (NHEJ)**
What happens is that in human cells, the repair mechanism has certain proteins that use the complementary chromosomes. As we know the cell is diploid (2n) , so let us assume that the dad chromosome is cleaved . The system utilizes or takes advantage of the homologous chromosome to fill the gaps , so that we can have the damaged chromosome repaired.	Here you do not have the use of homologous chromosome rather what happens is that you have proteins that glue the broken DNA together (ligation). However, it could be prone to errors leading to mutations.

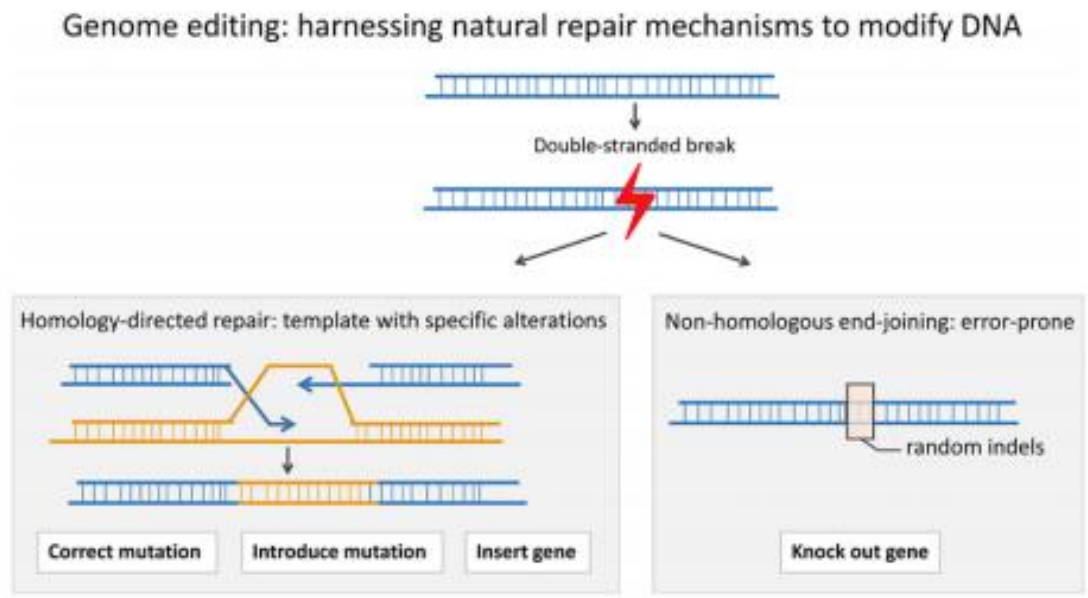


- **The steps of Action :-**

  - The guide RNA contains a region that is complementary to the DNA. This guide RNA binds to Cas9 and then Cas9 can go to a specific region in the DNA so that it can cleave it , allowing us to repair the damage or perhaps introduce a new gene.
  - Both the gRNA and CAS9 gene can be introduced in human cells as genes cloned into plasmid vectors

Fun-fact:

The idea of utilizing CRISPR-CAS9 system was by Two scientists who came up in this idea and got the Nobel prize in 2020. In fact, CRISPR/ Cas9 system was discovered by a Portuguese scientist in bacteria except that his thinking did not go beyond what the CRISPR/Cas9 does in bacterial cells.



The idea is that they would introduce breaks into the DNA by Cas9 and you will have activation of both or one of the DNA repair systems.

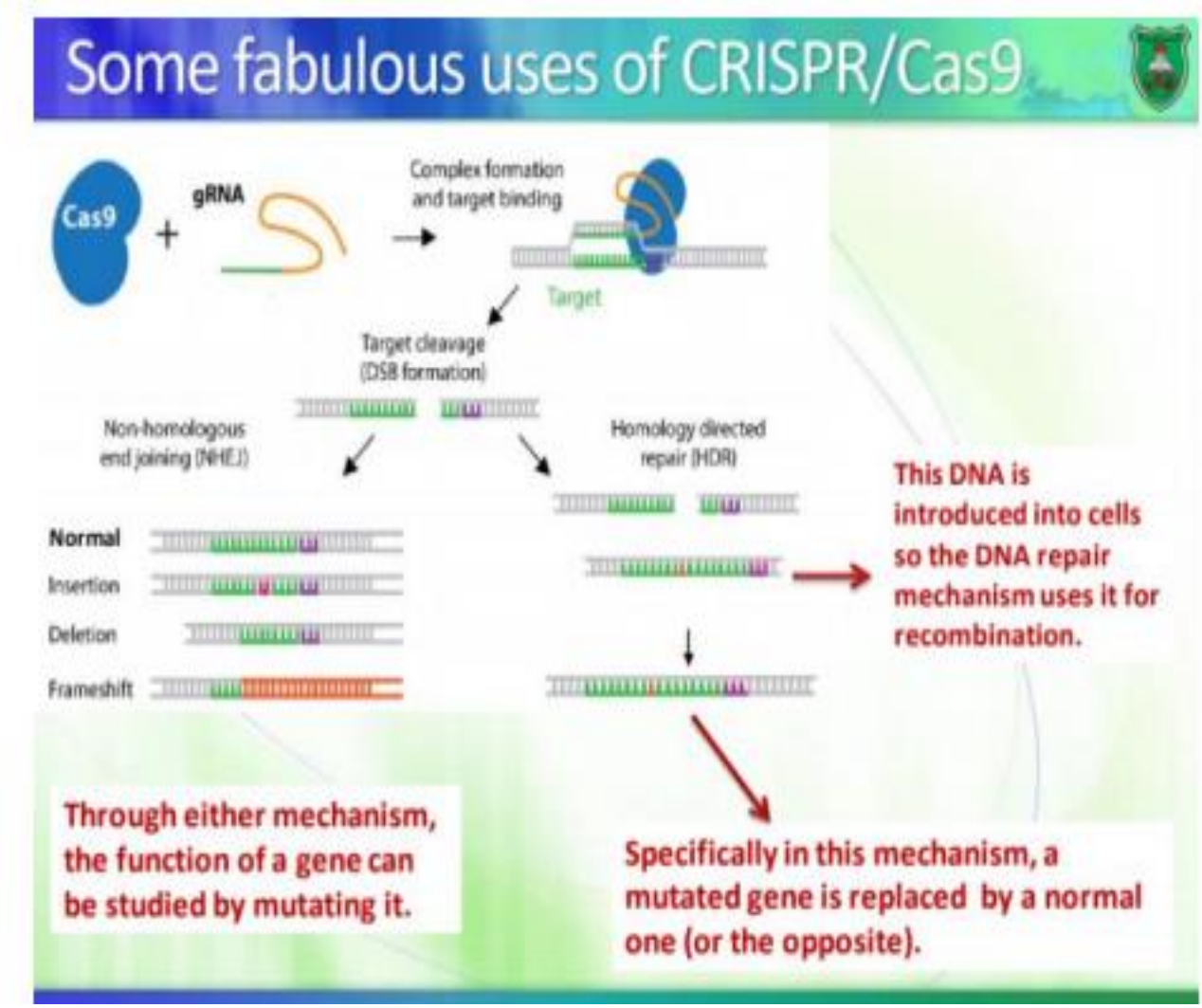
- Scientists have noticed that if there is activation of the **non-homologous end joining (NHEJ)** system and if you have gluing of the DNA, you will have introduction of indels.

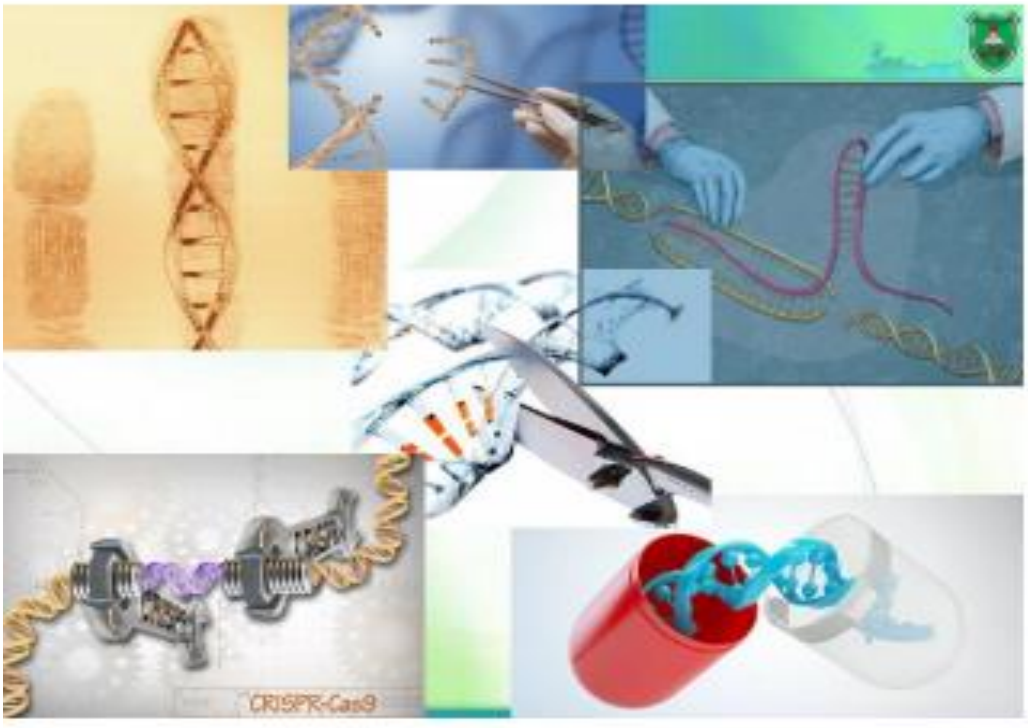
Indels stand **for insertion-deletion mutations**. So, if you have a gene right here and you have insertion or deletion, you are knocking out the gene/ disrupting the gene and it becomes non-functional.

- -Now if you have activation of the **homology repair system** (we have homologous chromosome in our cells) you would have replacement of the damaged DNA and it would be replaced by fragments from the homologous chromosome. And as a result of which you can many outcomes :
  - If you have a mutated gene , you can repair it by utilizing a correct homologous chromosome .
  - it can be the opposite. You can have a good gene and what you do is replacing it by a mutated DNA or gene.
  - Finally , you can insert a whole new gene thereby introducing different outcomes .

Notes in the previous two methods :

- **NHEJ** is less accurate than **HR** as NHEJ is prone to errors and could lead to mutations .
- When the DNA is cut , it could produce either **Cohesive ends** and in this case the **HR** system will be activated , or it could be **blunt-ended fragments** and in this case the **NHEJ** system will be activated.
- **The CAS9-CRISPR System** is transfected into human cells from bacteria via the use of vector plasmids. However, Recombinant repairing systems occur only in the human cell.
- When scientists intend to modify a certain gene, they insert CAS9-CRISPER along with a DNA piece to work as a template in HR (DONOR-DNA) so that they can control what gene will be inserted in the damaged DNA as well as changing sequencing to what they want.





So again , A quick recap to what the CRISPR-CAS9 system really does :

Basically you can introduce Cas9 and the guide RNA into human cells. The guide RNA takes Cas9 to a certain region in DNA and Cas9 cleaves the human DNA.

You have the activation of the non-homologous end joining and you can introduce indels insertion or deletion mutations within the DNA and that eventually leads to frame shift mutation if we are talking about a gene that is mutated.

Now if you have activation of the homology repair system, you can introduce a new piece of DNA and you can correct a mutation or even you can introduce a mutated piece of DNA.

This is really the act of manipulating DNA.

You can insert a piece of DNA of your choice, you can do genetic engineering, you can play with DNA by cutting and modifying it and you can introduce new pieces of DNA into our cells

Self-assessment quiz :-

1. The Underlined “R” in the word CRISPR refers to:

a) repeats b)regularly c) RNA d)none of these are correct

2.Through both HEJ and NHEJ, the function of a gene can be studied by..... it

a) exonuclease b)endonuclease c)mutating d) none of these

3 . In NHEJ we take the advantage of the cell being diploid

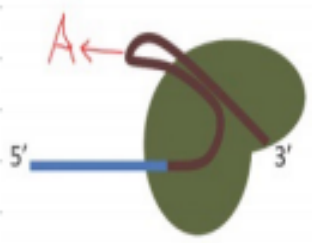
A) true. B) false

4. The CRISPR function in bacteria is acting as immune system

A) true. B) false

5. The RNA which is referred by the letter “A” is :

a) mRNA b) gRNA c) sgRNA d) b and c are correct



>> B C B A D

The End