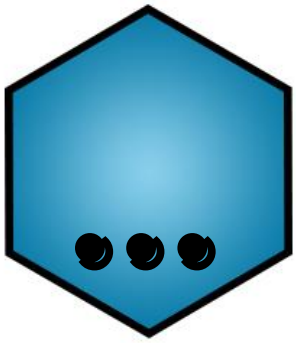

Faculty of medicine – JU2018

◉ Sheet ○ Slides

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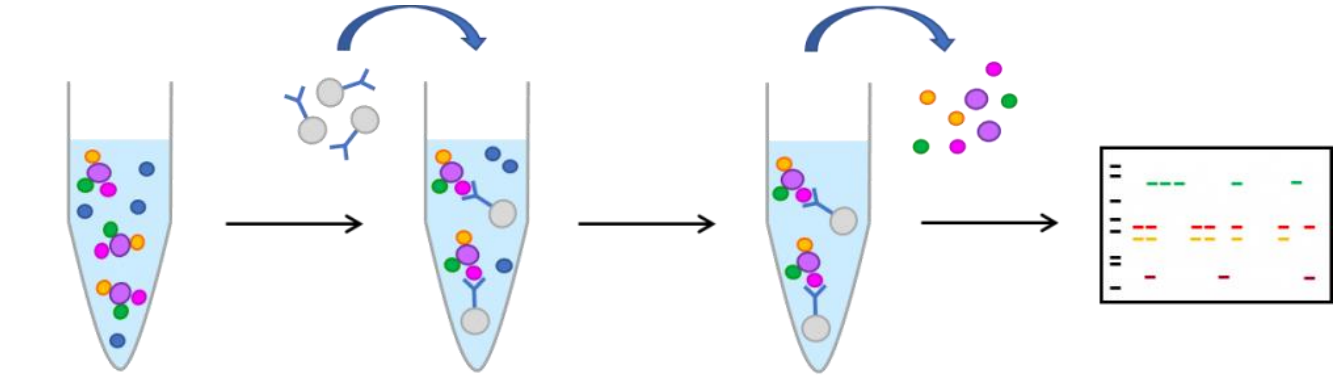
Protein-Protein interaction

Introduction:

Proteins control all biological systems in a cell, and while many proteins perform their functions independently, the vast majority of proteins interact with others for proper biological activity. In the recent past, the researchers have shown interest in the analysis of protein-protein interaction because they wanted to understand how proteins interact with each other and what proteins do so. One of the common methods that was used to analyze the various types of protein interactions is known as (Co)-Immunoprecipitation.

Co-Immunoprecipitation:

In this technique we use beads to which antibodies are attached, these antibodies can bind specifically to our protein of interest in the test solution, when the protein binds to the antibody on the bead, it precipitates along with the co proteins (proteins that interact with our protein of interest), which makes it easier for us to separate it from the rest of the solution.



Immunoprecipitation Vs. Co-Immunoprecipitation:

The Co-Immunoprecipitation is based on the same methodology as immunoprecipitation in its ability to separate and purify the protein of interest, however Co-Immunoprecipitation is focused on the additional proteins that interact with our protein of interest.

Upstream Activating Site (UAS), LacZ gene and X-gal:

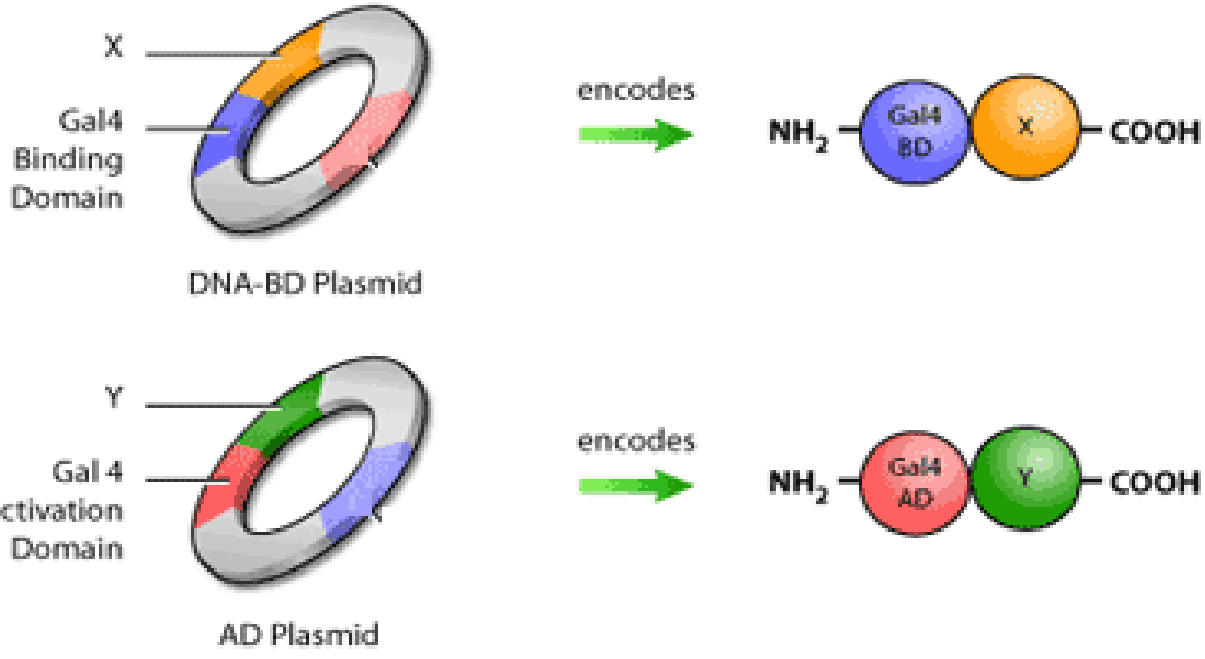
We want to know what proteins interact with the protein of interest, to do that so, we must grasp two concepts:

- **UAS (Upstream Activating Site):** it's a part of the promotor region in yeast cells, it activates the transcription of the following genes by the binding of a transcription factor to it. This transcription factor is made up of two domains (they fold and function independently) a DNA binding domain (BD) and an activating domain (AD). If these two domains are close to each other, the following gene will be transcribed.
- **LacZ gene and X-gal:** LacZ gene is a bacterial gene that produces B-galactosidase which cleaves Lactose. X-gal is the substrate we use to grow the yeast colony in. When B-galactosidase cleaves X-gal, a blue color is produced.

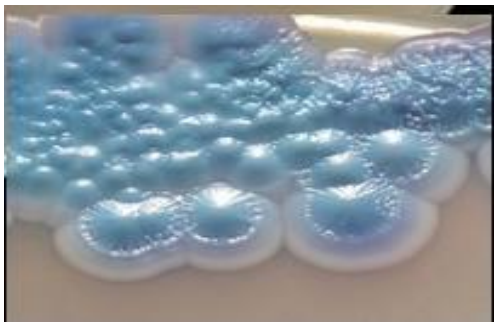
Procedure:

1- We introduce a recombinant plasmid to the yeast colony; this plasmid has the UAS followed by the LacZ gene (reporter gene).

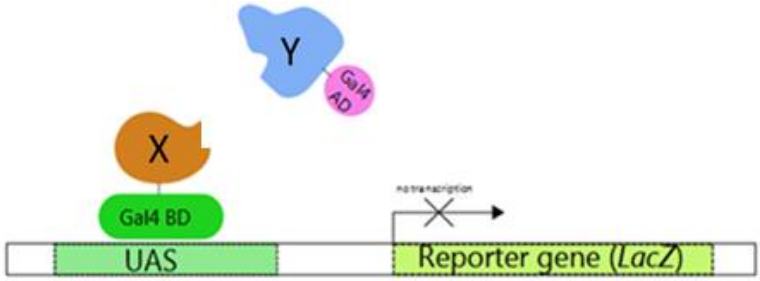
2- We create two vectors by hybridizing one domain of the transcription factor (for example, the DNA binding domain) to our protein of interest (protein X), and the other domain to the protein we want to test (protein Y).

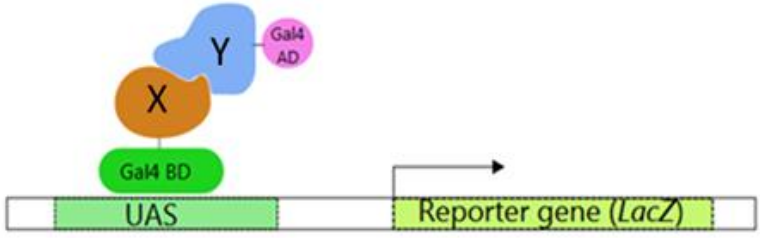


3- If protein X binds to protein Y then the two domains of the transcription factor will be near each other, so the UAS will activate the transcription of the LacZ gene; leading to the cleavage of X-gal producing blue stained colonies.



4- If protein X doesn't bind to protein Y, then the transcription factor won't activate the UAS and the LacZ gene won't be transcribed so the yeast colonies will appear white.





Summary:

1. UAS system **active** -> Expression of LacZ gene is active -> Production of beta galactosidase is on -> beta galactosidase cleaves X-gal producing a blue pigmentation in the yeast colonies
2. UAS system **inactive** -> Expression of LacZ gene is OFF -> production of beta galactosidase is OFF -> x-gal is NOT cleaved-> colonies would have a white colour

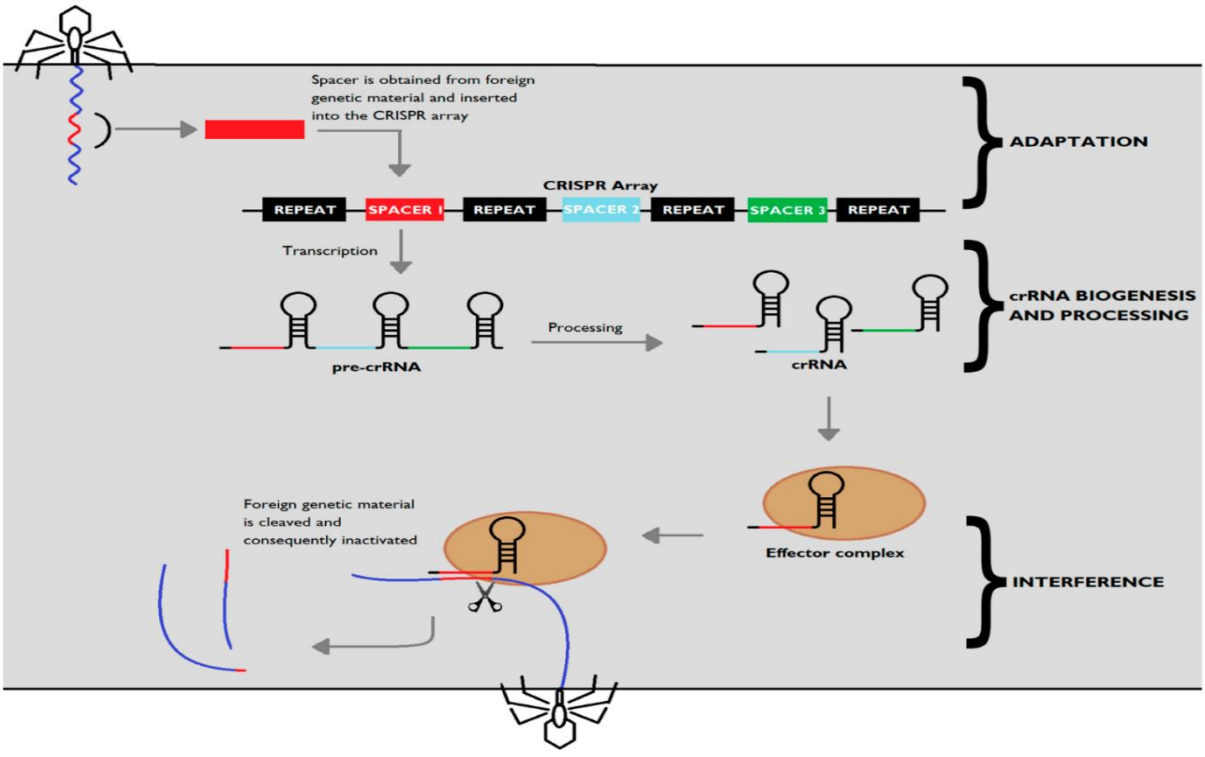
CRISPR-CAS9:

-Bacteriophages (bacterio- = related to bacteria, -phage = eat or devour) are viruses that devour bacterial cells, but as humans develop immunity against diseases, bacterial cells can develop immunity against bacteriophages.

The immune system of the bacteria is made up mainly of two parts:

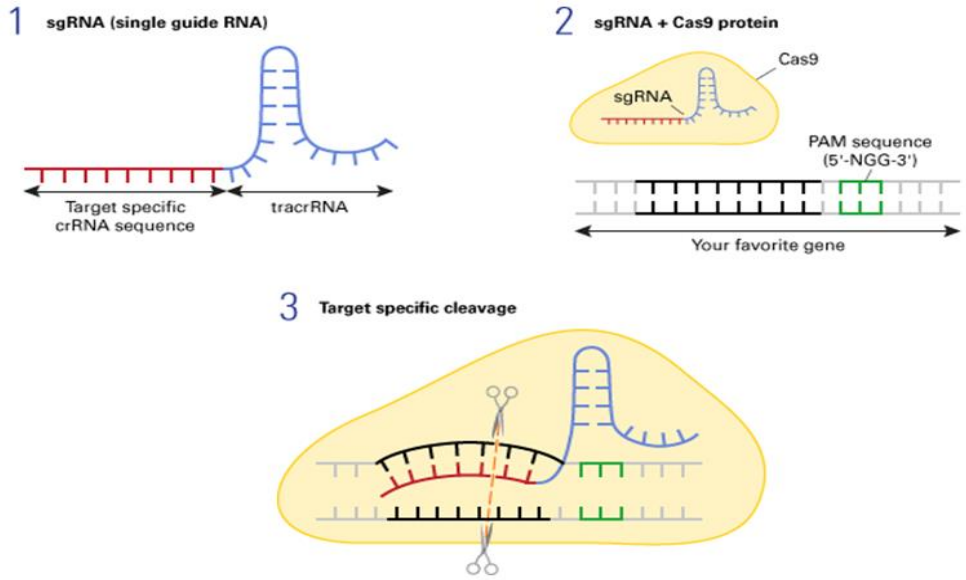
1- CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats.
2- CAS9: (CRISPR ASsociated protein) An RNA guided endonuclease that can either create single or double stranded breaks.

When a bacteriophage enters a bacterial cell, the bacteria cleaves the genetic material of the bacteriophage into smaller fragments (spacers) and inserts these fragments between certain repeats in the bacterial DNA, so we end up with a **Cluster** of **Short Repeats** that are **Regularly Interspaced** by bacteriophage's DNA fragments (spacers) in a **Palindromic** manner (repeat – spacer – repeat – spacer -… meaning that the sequence is read the same whether from {5' to 3'} or {3' to 5'}) , thus CRISPR.



When the bacterial cell is infected by the bacteriophage again, the bacteria transcribes the CRISPR region into RNA producing guide RNA (gRNA). The gRNA is complementary to the bacteriophage DNA.

The gRNA then binds to cas9 and guides it to the bacteriophage's DNA that has been placed in the bacterial genome, once the gRNA recognizes the complementary DNA sequence, the cas9 makes a single strand cut or a double strand cut, disabling the viral DNA.

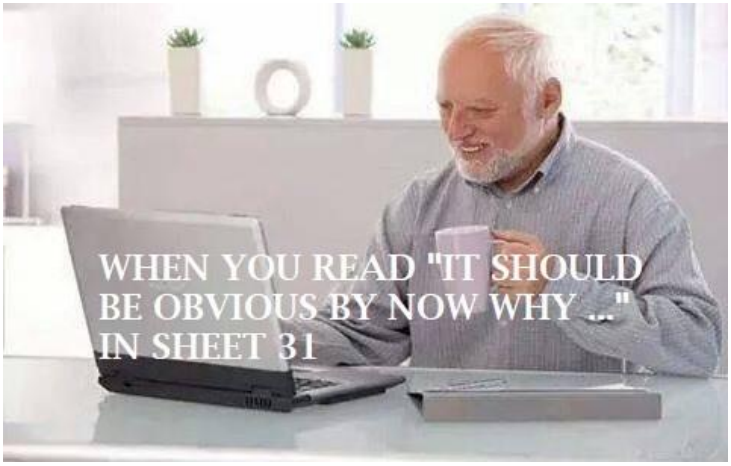


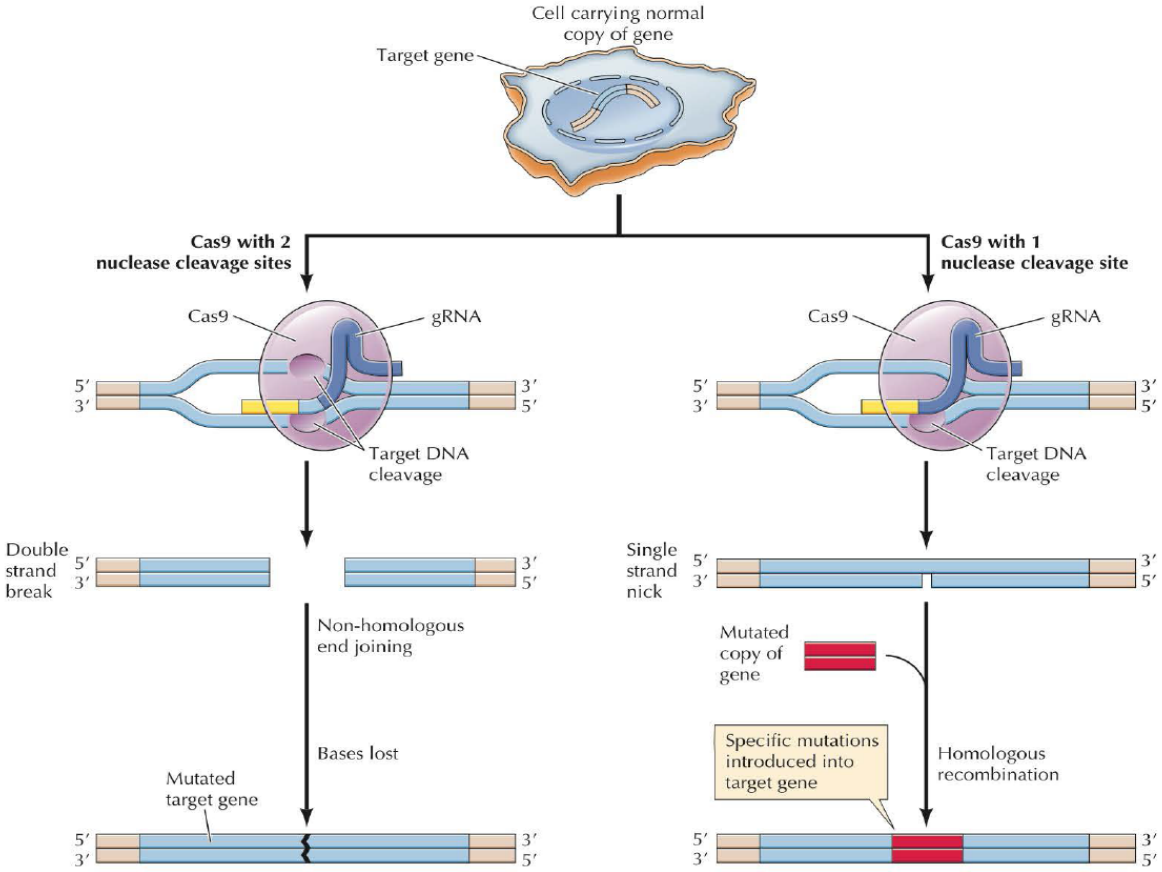
The CRISPR-cas9 system can be introduced into the human cells, and when the cas9 breaks the DNA, the human cells will try to repair that cut by one of these two repair mechanisms:

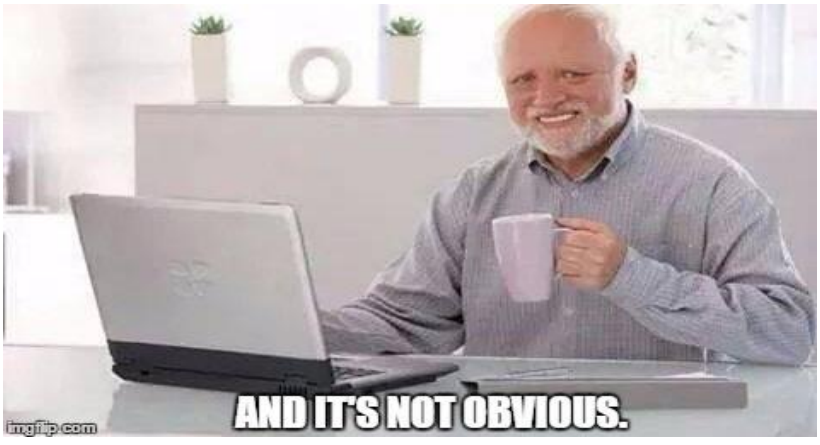
1- **Non homologous end joining**: the cells will use this repair system when the cas9 does a double strand cut, the cells will reattach the ends of the cut, but in this process some bases will be lost, introducing mutations to the gene.

2- **Homologous recombination**: the cells will use this repair system when the cas9 does a single strand cut, the cells will remove the site with the cut and will try to repair it by replacing the broken part with a normal one. We can use this method to repair mutated genes by putting a normal gene in the cell via a recombinant plasmid, and once the cas9 makes the break the cell will replace the broken part by the normal part from our plasmid.

(Please refer to the image in the next page, it should be **obvious** by now)


WHEN YOU READ "IT SHOULD
BE OBVIOUS BY NOW WHY ..."
IN SHEET 31


Cell carrying normal copy of gene
Target gene
Cas9 with 2 nuclease cleavage sites
Cas9 with 1 nuclease cleavage site
Cas9
gRNA
5′
3′
3′
5′
Target DNA cleavage
Double strand break
Single strand nick
Non-homologous end joining
Bases lost
Mutated target gene
Mutated copy of gene
Specific mutations introduced into target gene
Homologous recombination


AND IT'S NOT OBVIOUS.
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