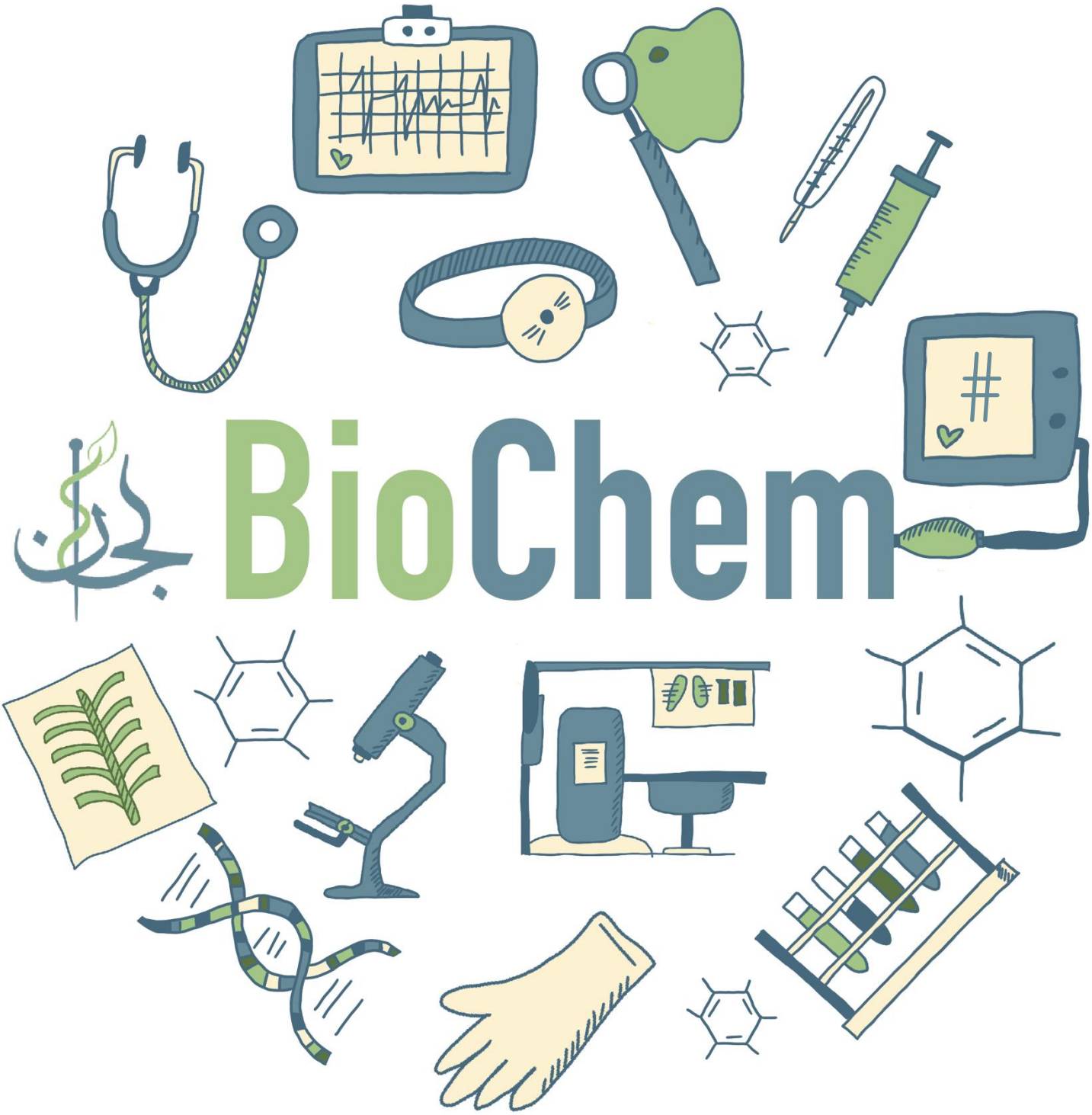

BioChem

جبن

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Some important concepts:

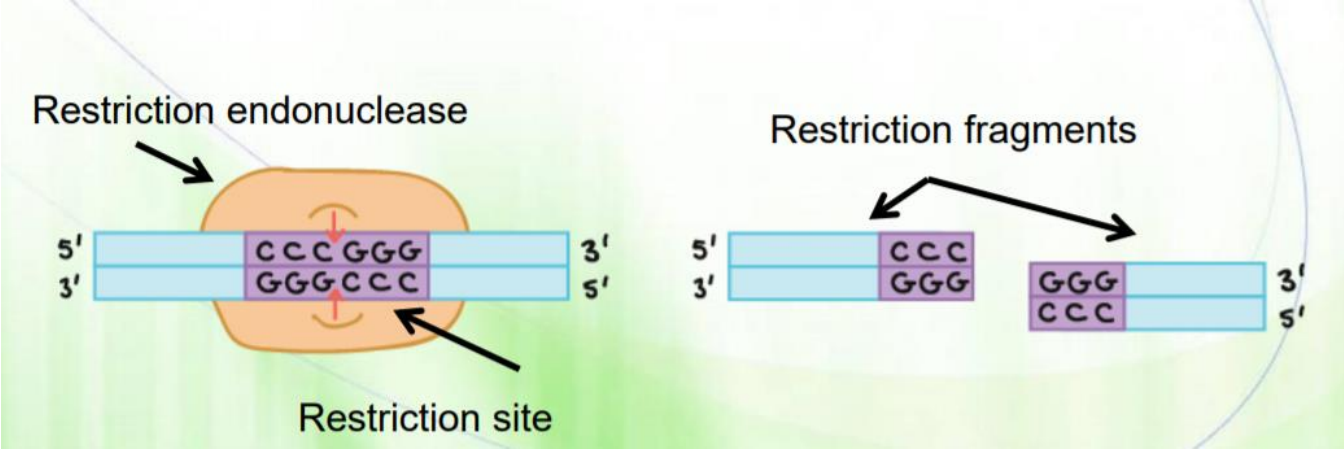
Nucleases are enzymes that cleave nucleic acids (DNA, RNA) specifically we have:

1-Endonucleases that cut DNA in the middle of the molecule

2-Exonucleases which cleave the DNA from the end of the molecule

Restriction Endonucleases: bacterial enzymes that recognize and cut (break) the phosphodiester bonds between nucleotides at specific sequences (4-8 bp restriction sites) generating restriction fragments.

For example, in this figure restriction endonucleases recognized specific sequences and cut between C and G to generate restriction fragments.



Important:

If the sequence recognized by the restriction endonuclease are read the exact same way from 5' to 3' on both strands, then these sequences are called palindromic sequences.

Another way to define it is that the sequences recognized by restriction endonucleases—their sites of action—read the same from left to right as they do from right to left (on the complementary strand) are called palindromic sequences.

Examples of restriction endonucleases with palindromic sequences:

EcoRI	5'	GAATTC	3'
	3'	CTTAAG	5'
HindIII	5'	AAGCTT	3'
	3'	TTCGAA	5'
SmaI	5'	CCCGGG	3'
	3'	GGGCCC	5'

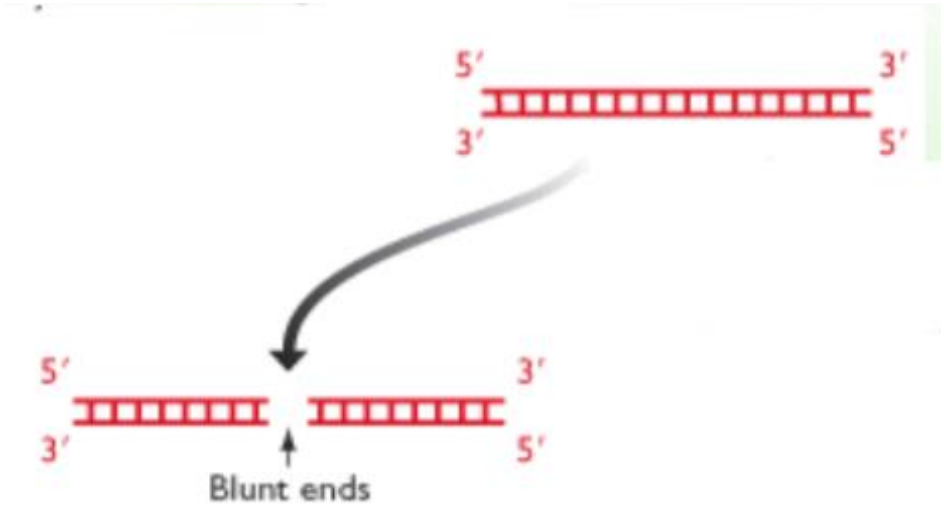
Each Restriction endonucleases recognizes specific sequence, for example:

ECORI recognizes the sequence GAATTC and so on.

Restriction Endonucleases cut DNA in two ways:

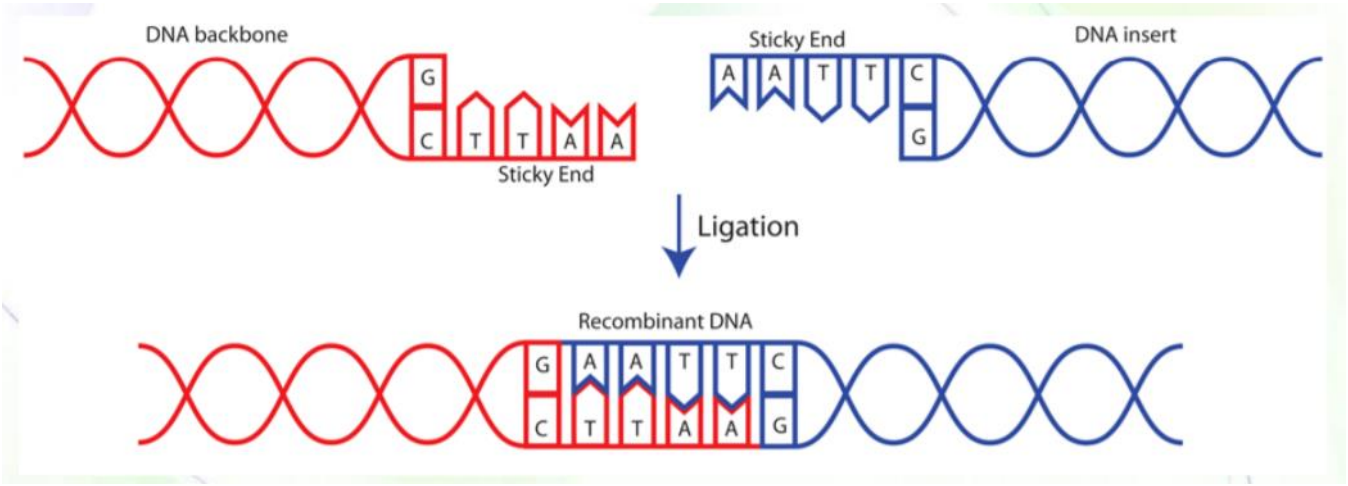
1.Blunt (sharp) the cut goes straight (the ends are smooth)

-Enzymes cut at the same position on both strands giving blunt ended fragments.



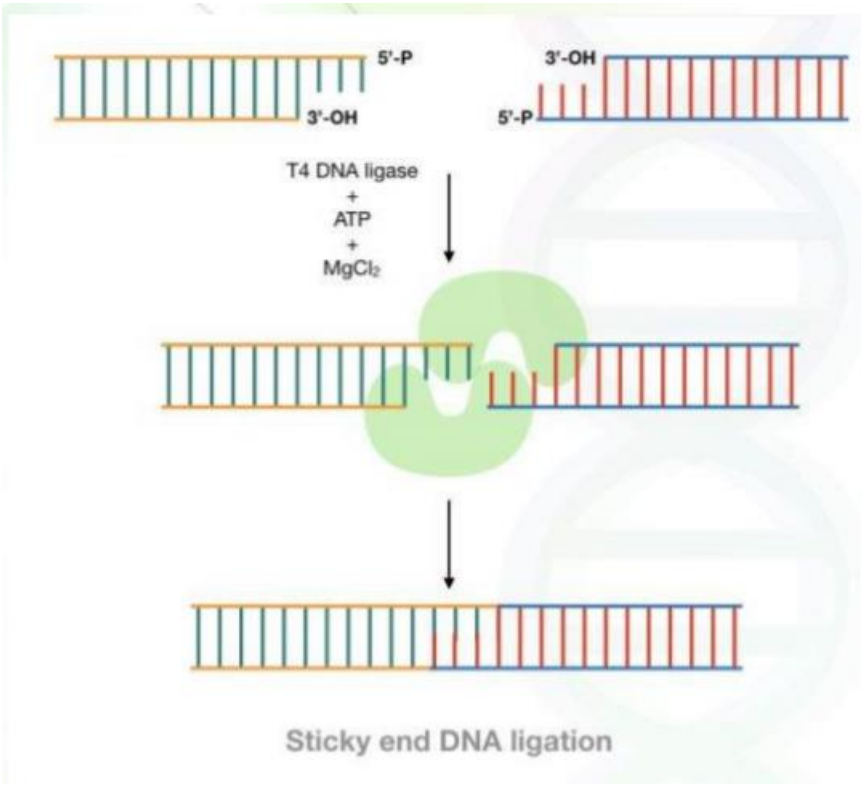
2.Staggered (off-center) enzymes cut the two DNA strands at different position generating sticky or cohesive ends

(for example: we have a cut between G and A in the first strand and on the other strand the cut occurs between the G and A resulting in the formation of sticky ends we called them "sticky ends" because the ends are complementary to each other which mean the ends can form hydrogen bonds between each other except that they are not stable (why?) because there are no phosphodiester bonds between nucleotides.



We can form phosphodiester bonds between the G and A by an enzyme known as DNA ligase this action is called ligation (gluing) of fragments with each other to become stable.

It covalently joins DNA ends (like restriction fragments) by catalyzing the ATP – dependent formation of phosphodiester bonds between the 3' hydroxyl group of one strand and 5' phosphate end of another strand.

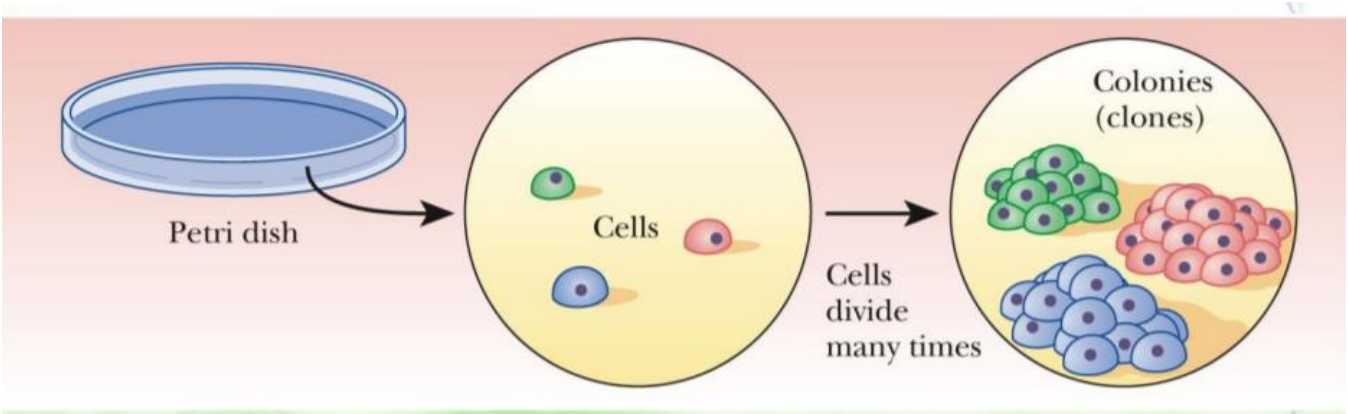


DNA Cloning

Another use of restriction endonucleases is known as cloning which means that you make several copies of one thing (if I say I want to clone DNA, it means that I want to make multiple copies of this DNA fragment)

A clone is genetically identical population whether of organisms, cells, viruses, or DNA molecules.

Every member of the population is derived from a single cell, virus, or DNA molecule.



Cloning is really something natural in bacteria because we can have the bacterial cells in the plate (petri dish). One single bacterial cell will eventually after 24 hours form a

colony, each colony is originally cloned of one particular cell (these cells are identical because they have the same DNA sequence)

How do we clone a DNA molecule?

A DNA fragment of interest is inserted into a carrier (called a vector) that can be replicated.

The resulting DNA molecule is what known as a recombinant DNA molecule.

We use a carrier and this carrier usually is a DNA plasmid (extra chromosomal DNA pieces which is separate from the main circular bacterial DNA.)

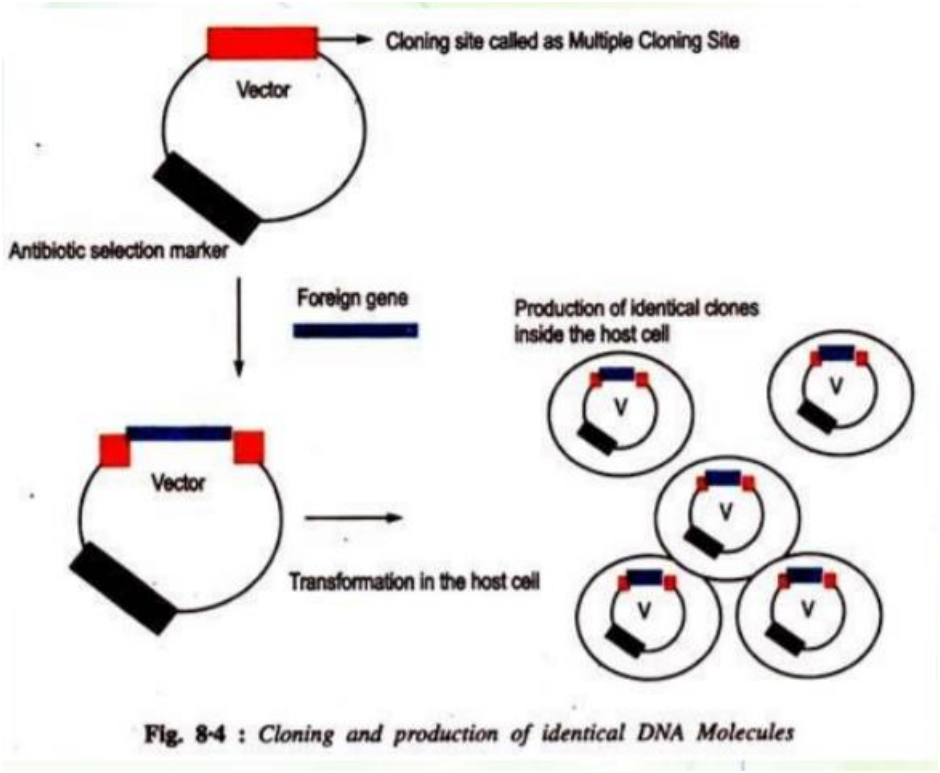
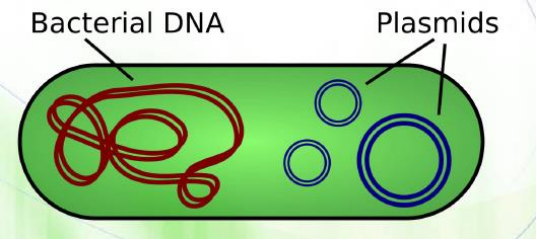


Fig. 8-4 : *Cloning and production of identical DNA Molecules*

Important Note: the bacterial cells have one chromosome but they can also have many copies of plasmid that can replicate independently of the bacterial chromosome.

Bacterial cells may have multiple plasmid (circular and small DNA) that contain some genes these genes give advantage to these bacterial cells.



We take a plasmid and insert the DNA fragment inside the plasmid so we have a DNA molecule having two different independent pieces of DNA integrated to each another. This is known as a recombinant DNA molecule.

The reason why we do this is that when we insert a DNA fragment into the plasmid, the plasmid is then inserted into bacteria and bacterial cells make multiple copies of same plasmid (basically we let bacteria clone the DNA molecule)

Using plasmids as vectors

Bacterial cells can have one chromosome and multiple plasmids as we said before.

These plasmids are bacterial circular DNA that is not part of the main circular DNA chromosome of bacterium.

A plasmid exists as a closed circle and replicates independently of the main bacterial genome.

Bacterial plasmids are considered excellent vectors.

Features of plasmids (most plasmids vectors contain at least three essential parts required for DNA cloning):

1.Can replicate independently of the bacterial chromosome and must have an origin of replication (ORI).

2.Can insert a foreign DNA fragment, this process is called recombinant DNA technology.

3.Can be selected for /against by an internal drug – resistance gene (selectable marker)

للتوضيح (For explanation)

If a bacterial cell has a plasmid with antibiotic resistance gene it don’t die or get killed by antibodies.

Making Recombinant DNA

1.Take a piece of DNA from a human cell then cut this DNA by a restriction endonuclease

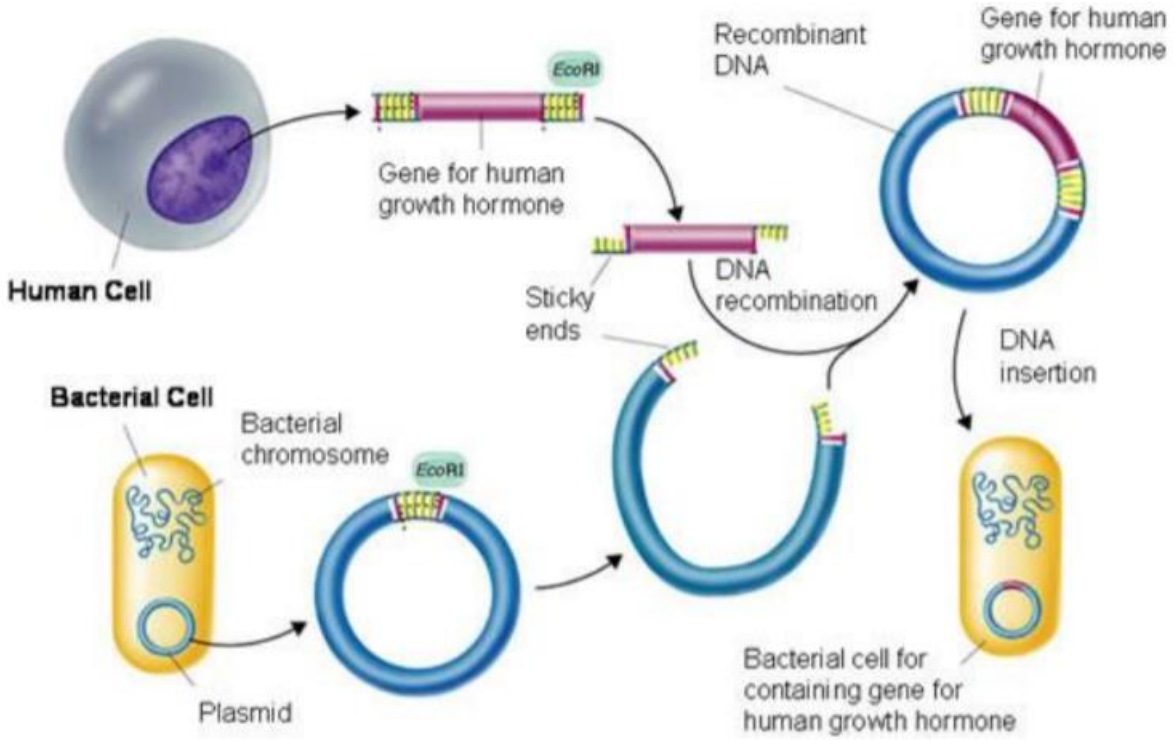
2.Cut a plasmid with the SAME restriction endonuclease

We use the same restriction endonuclease so we can have the same cohesive ends and they are complementary to each another (they hybridize with each other when mixed)

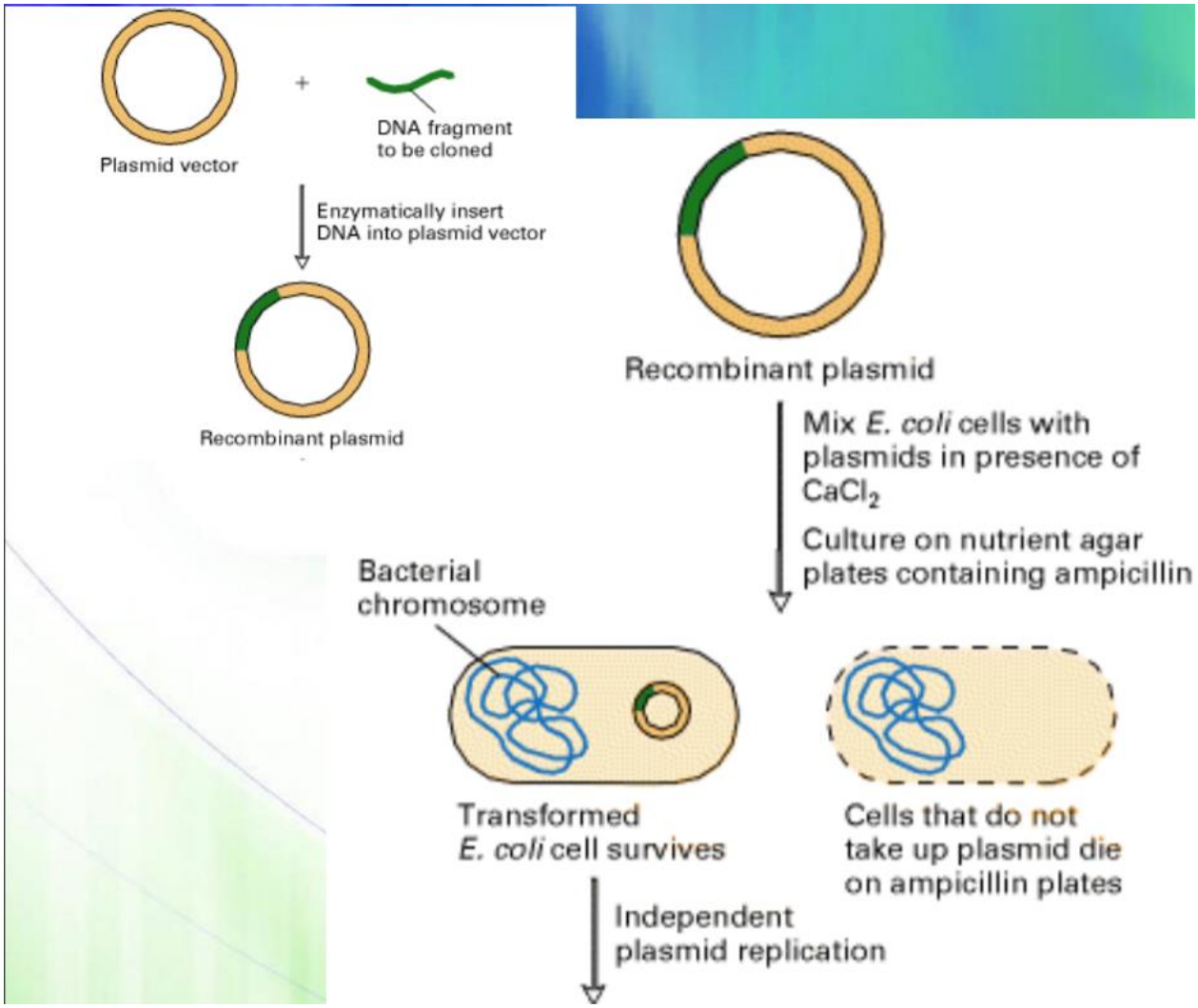
3.Combine human DNA with plasmid DNA to form plasmid DNA containing the human DNA fragment.

4.Add DNA ligase to make phosphodiester bonds creating a stable plasmid.

5.Finally, take this plasmid and put it back to the bacteria.

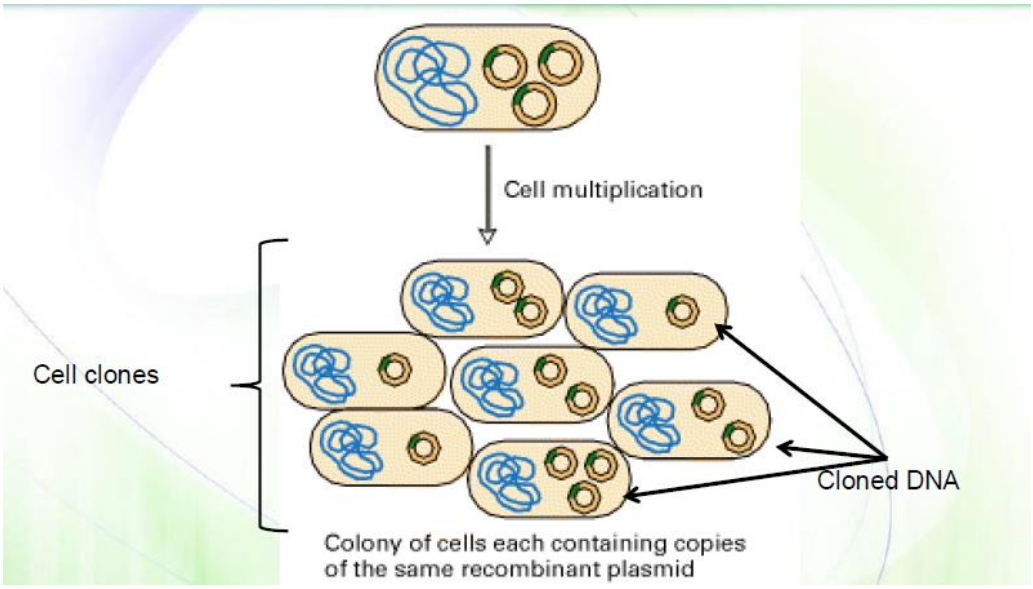


As we said before bacterial cell that don't have that plasmid would die because they wouldn't resist the antibodies.



A bacterial cell can have at least one plasmid ****

We can Imagine if we started with one bacterial cell that have one plasmid eventually after 24 hours we can have millions of bacterial cells and each one of them would have at least 1 plasmid (millions of copies of the same plasmid and that's how we clone DNA).



We can take advantage of plasmid by not only cloning DNA fragment we can also make bacteria express a gene (in another words making RNA which can be transcribed and translated into protein and this is used in medicine. For example: insulin (for people with diabetes), growth hormone, plasminogen activator (which is used in blood clotting), erythropoietin.

Expression Vectors

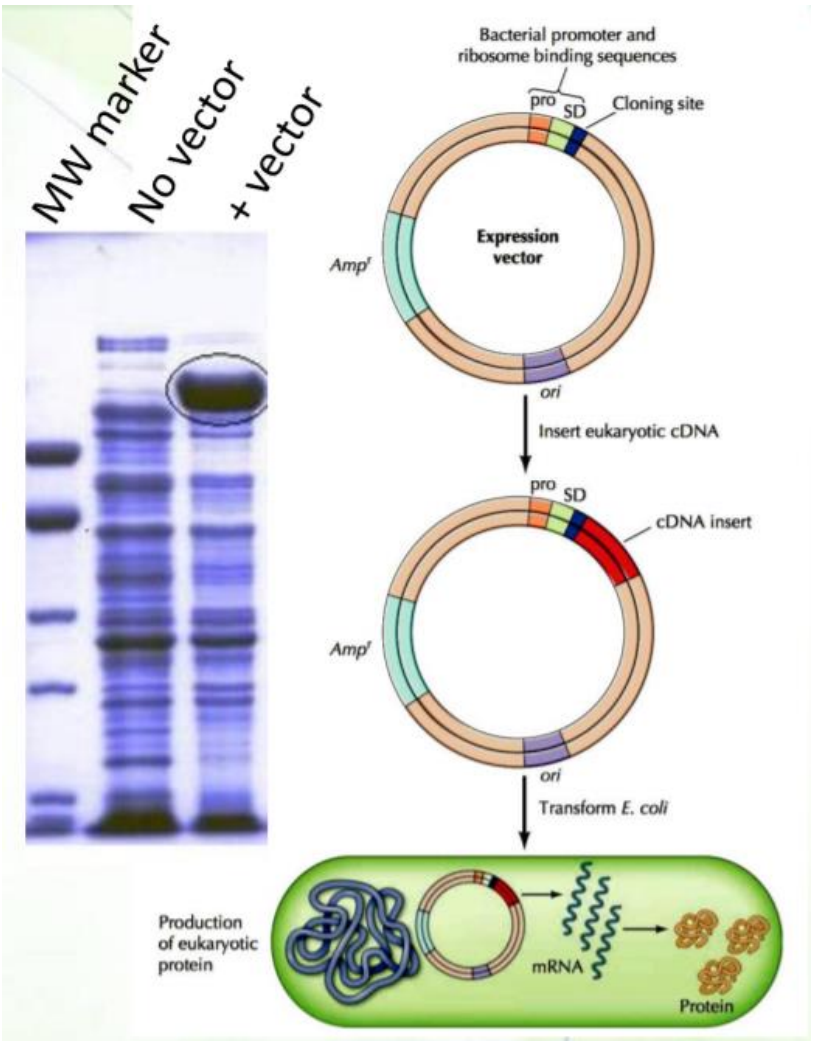
Expression vectors contain additional sequences in order to make bacteria produce protein:

1.Promoter sequences (region where the RNA polymerase binds to in order to start transcription) located upstream of gene (before it).

2.Ribosomal binding sequences (Shine-Dalgarno [SO] sequences)

3.Transcription termination sequence

The protein is expressed and then purified



Important: Challenges appear when producing human protein in bacteria:

1.Some human proteins have disulfide bonds (bonds between amino acids) but bacterial cells don't have these bonds (they don't have the enzymes to create them)

2 Proteins in human cells can be modified by for example glycosylation (addition of sugar to proteins) so some human proteins are known as glycoproteins and this can't occur in bacterial cells

3 Misfolding: Folding that is formation of protein with a functional structure may not happen in bacteria.

4.Degradation because bacteria recognize these proteins as foreign substances so they would degrade them.

Solution: use a eukaryotic system such as yeast because they are single cells and grow fast like bacteria.

Protein tagging or creation of protein hybrids

Another advantage of using cloning of a human gene into plasmid what is known as protein tagging (basically addition of certain sequence to a protein). HOW??

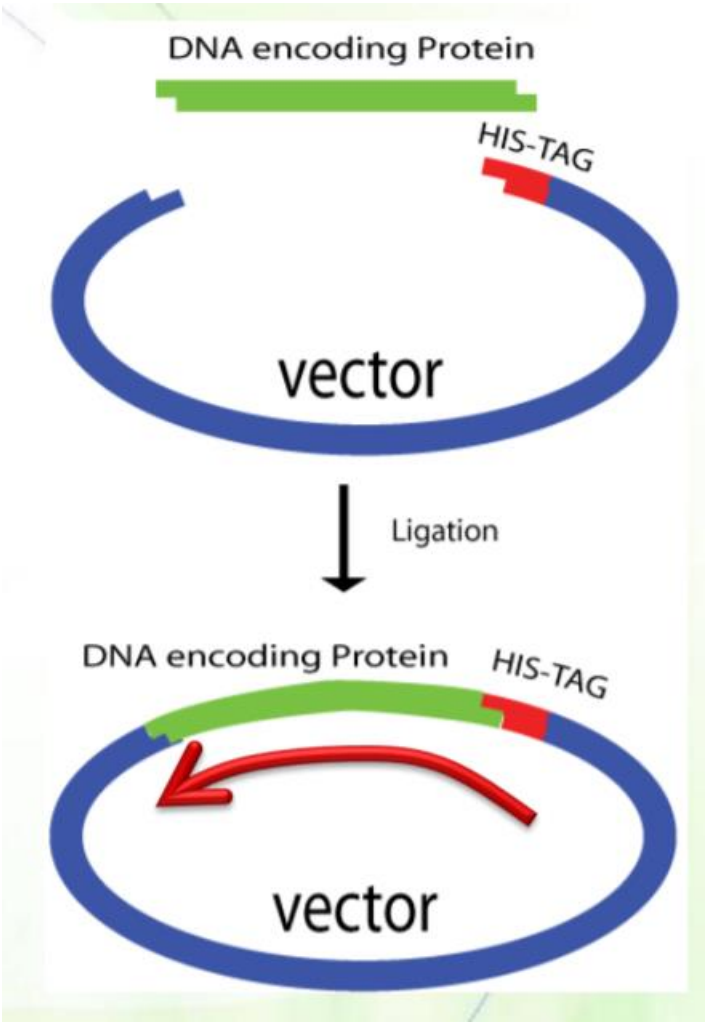
Additional sequences are added to the plasmid (these sequences are called tags)

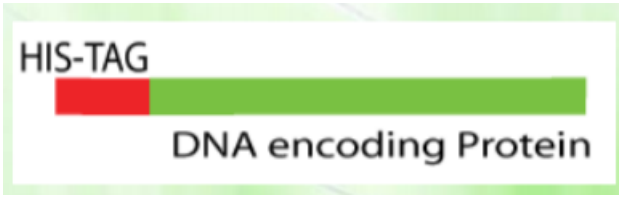
When bacteria do transcription and translation, the protein that is produced would have the tag attached.

What is the important of tagging?

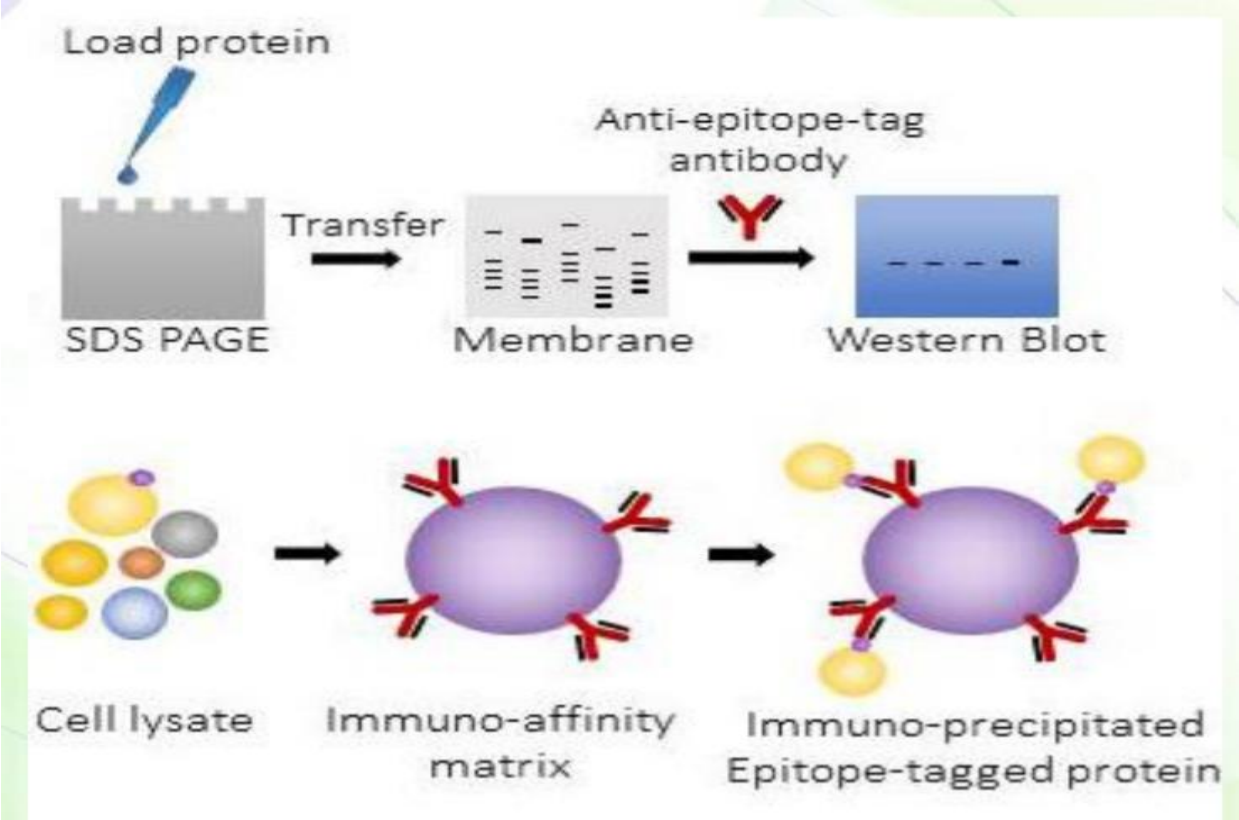
These tags allow for easy protein purification and detection

We have small tags such as six histidines attached to a protein and some tags are large proteins (in this case we would have two proteins link to each other, proteins of enhancers and as well as another protein (GFP, GST)).





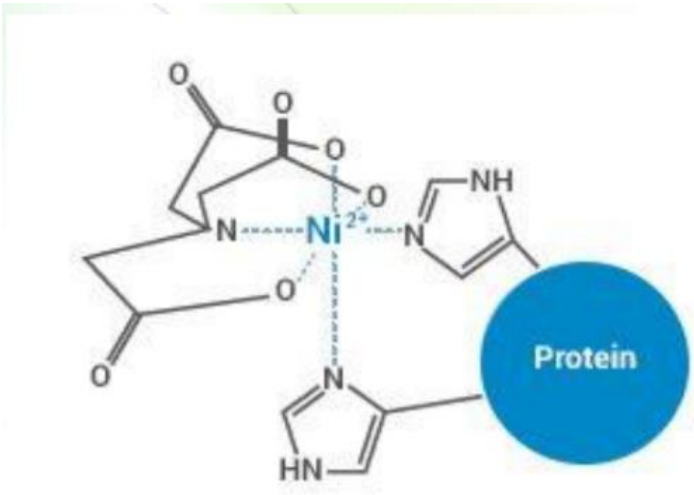
Name	Amino acids	Detection	Purification
FLAG	DYKDDDDK	antibody	FLAG peptide
Green fluorescent proteins (GFP)	~220 aa protein	antibody or fluorescence	None
Glutathione S transferase (GST)	218 aa protein	antibody	glutathione
HA	YPYDVPDYA	antibody	HA peptide
Poly-His	HHHHHH	antibody	nickel, imidazole
Myc	EQKLISEED	antibody	Myc peptide
V5	GKPIPNPLLGLDST	antibody	V5 peptide

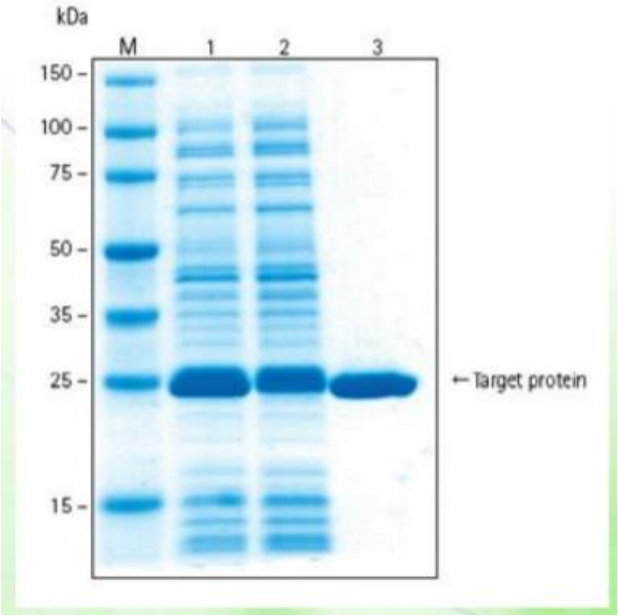


His tag:

The addition of six-histidines to a protein would allow for purification using beads with bound nickel ions.

The important of six-histidines is that they can bind to a metal like nickel to isolate it from other proteins that don't bind to the nickel, as we will show in the figure below:

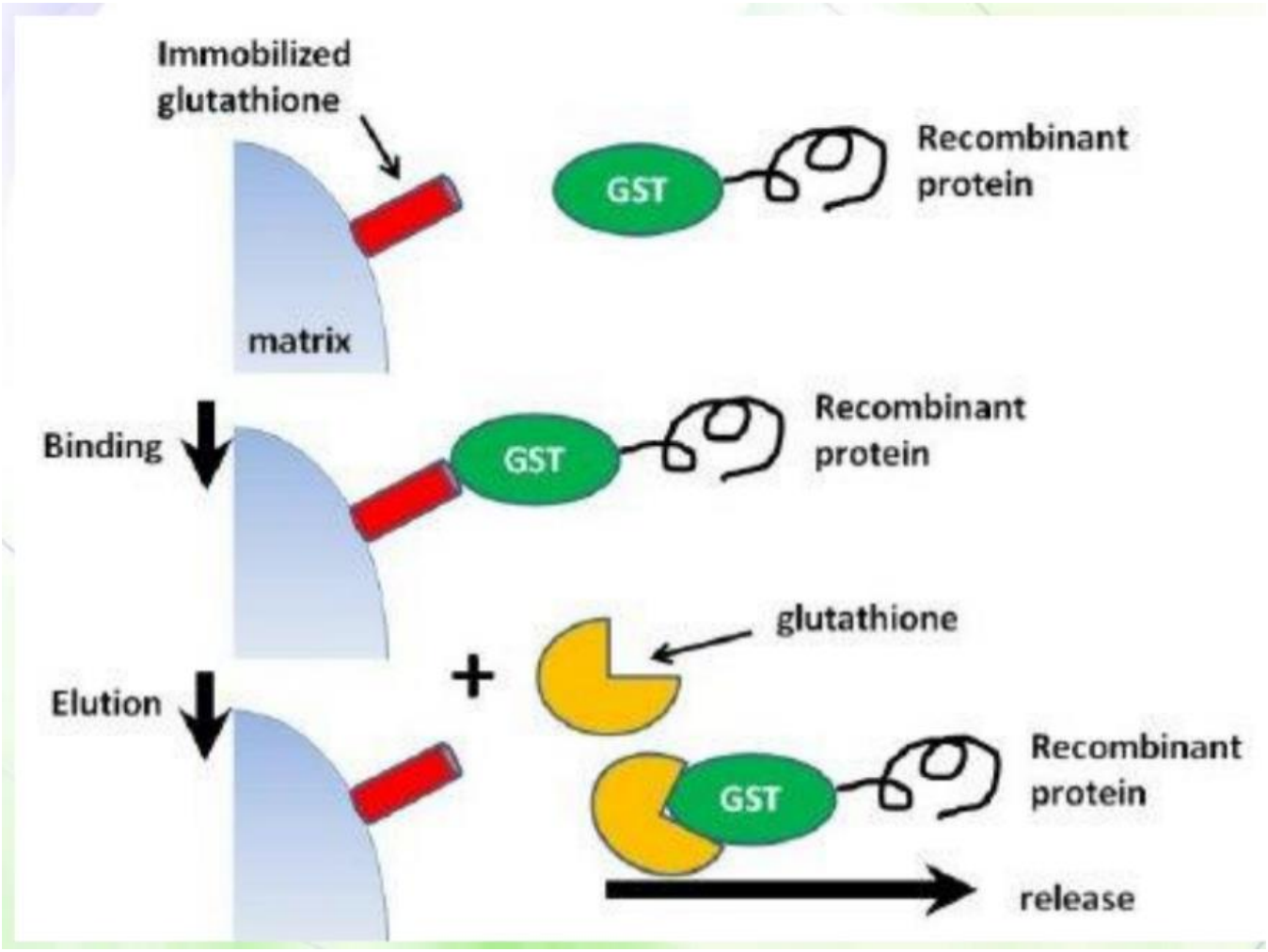




Purification of GST tagged proteins:

Protein hybrid: a protein that consists of multiple different proteins.

Glutathione S Transferase (GST) is an enzyme that binds to a specific substrate which is glutathione (a tripeptide).



We can design beads with attached glutathione residues, and when our sample passes through the beads, only GST-tagged proteins will attach to the beads, other proteins are washed away.

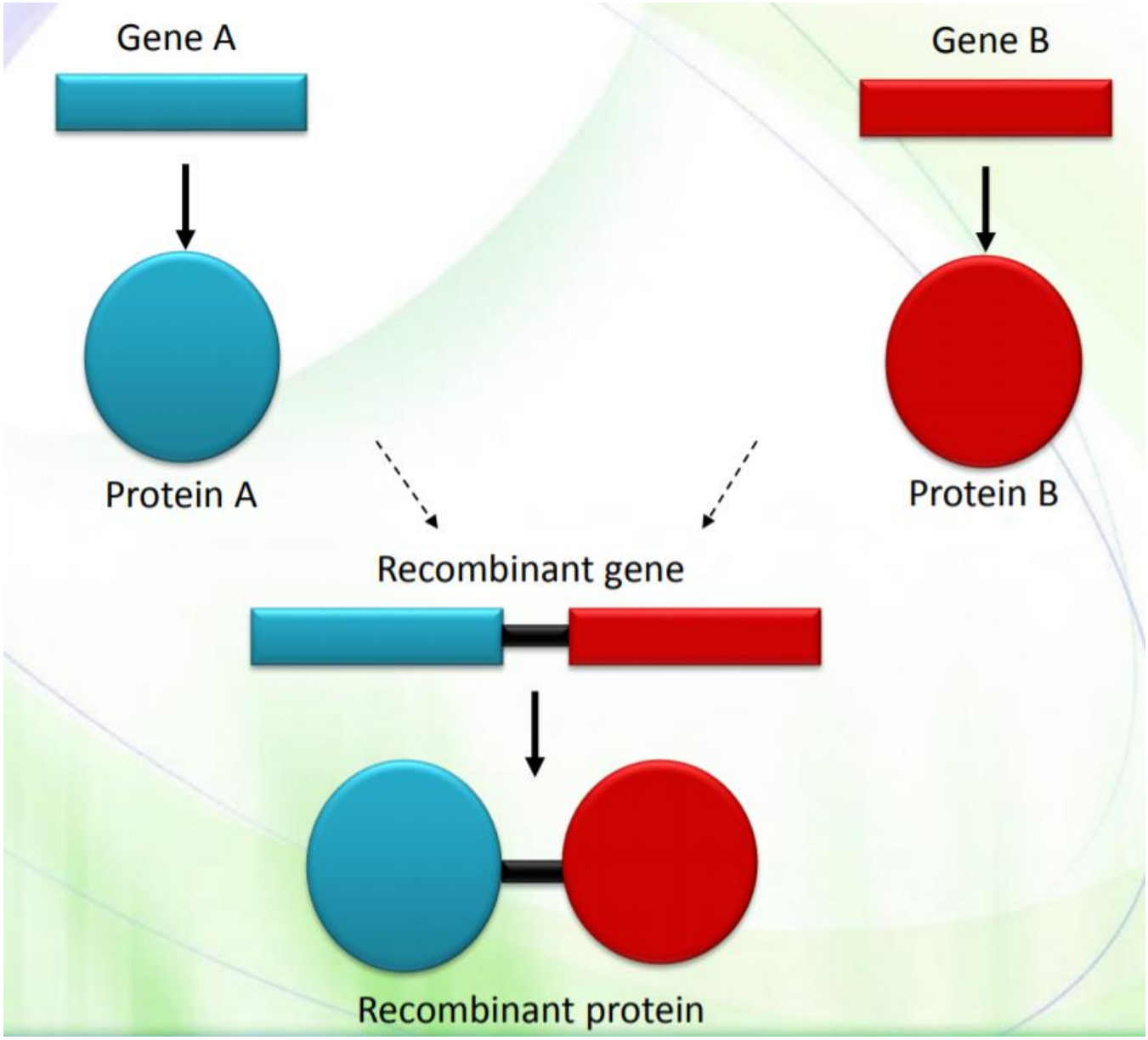
Then, we can release the GST-tagged Proteins by adding free glutathione so the that the GST-tagged proteins bind to it and move along with it and are then collected.

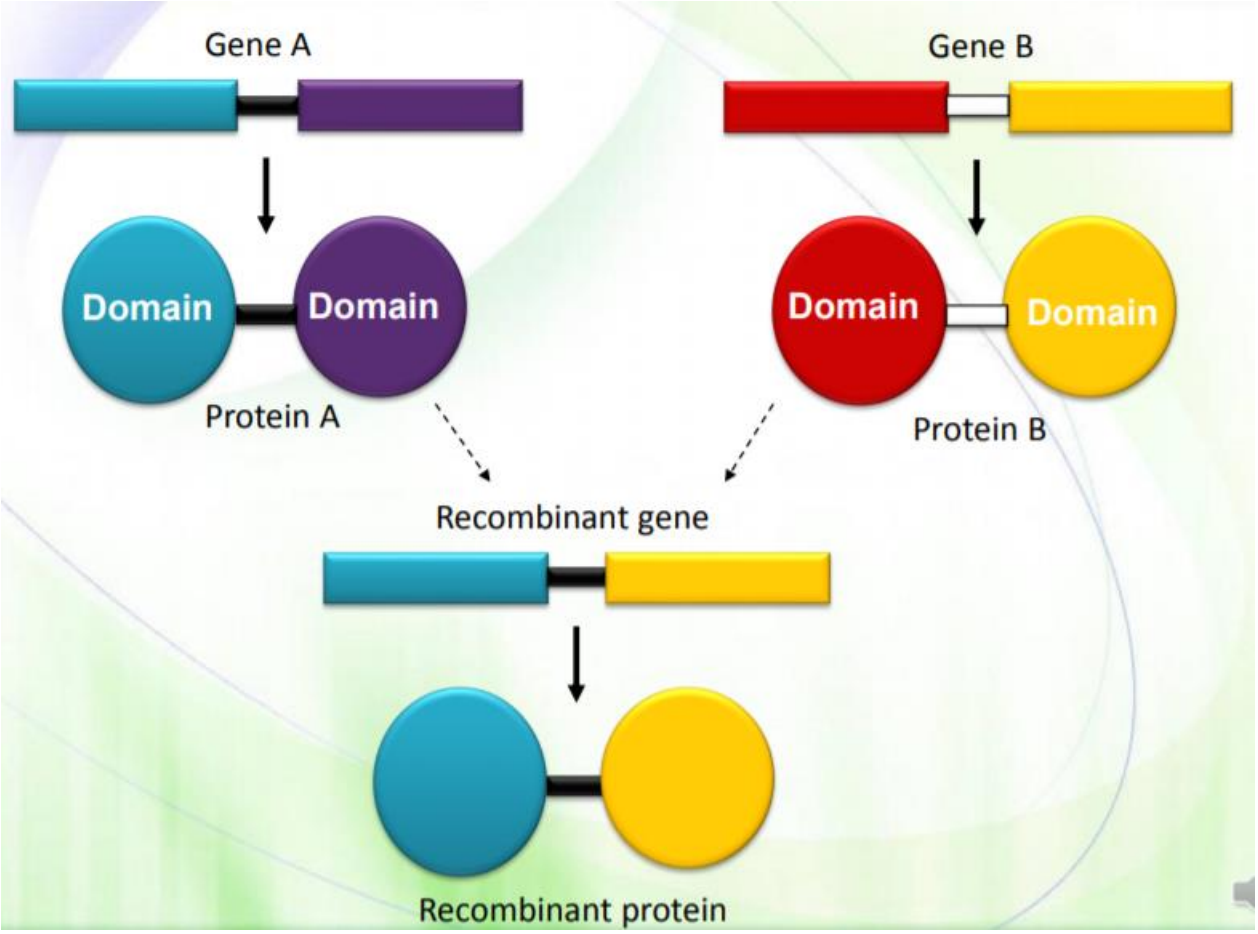
How can we produce a large protein that has two different types of proteins?

We put gene A (known protein) and gene B (that I want to study further) together next to each other so when they are transcribed and translated they will produce one large protein linked to each other this is known genetic engineering.

Another example is that we can take on domain from a two domain protein and another domain from a different two domain protein and put them together to make a recombinant protein (this protein will have two domains each from a different protein)

IMPORTANT: Remember that each domain has a certain function





GFP-tagged proteins:

Another example of protein hybrids is: GFP-tagged proteins.

GFP (Green Fluorescent Protein): it is a protein that comes from jellyfish (these living creatures give a fluorescent color due to production of GFP) and scientists took advantage of this protein in tagging. They were also able to separate proteins with other colors later on.

The GFP portion of the GFP-tagged protein folds independently of the protein of interest, but is attached to it. (The same concept as domains). Both proteins maintain their function.

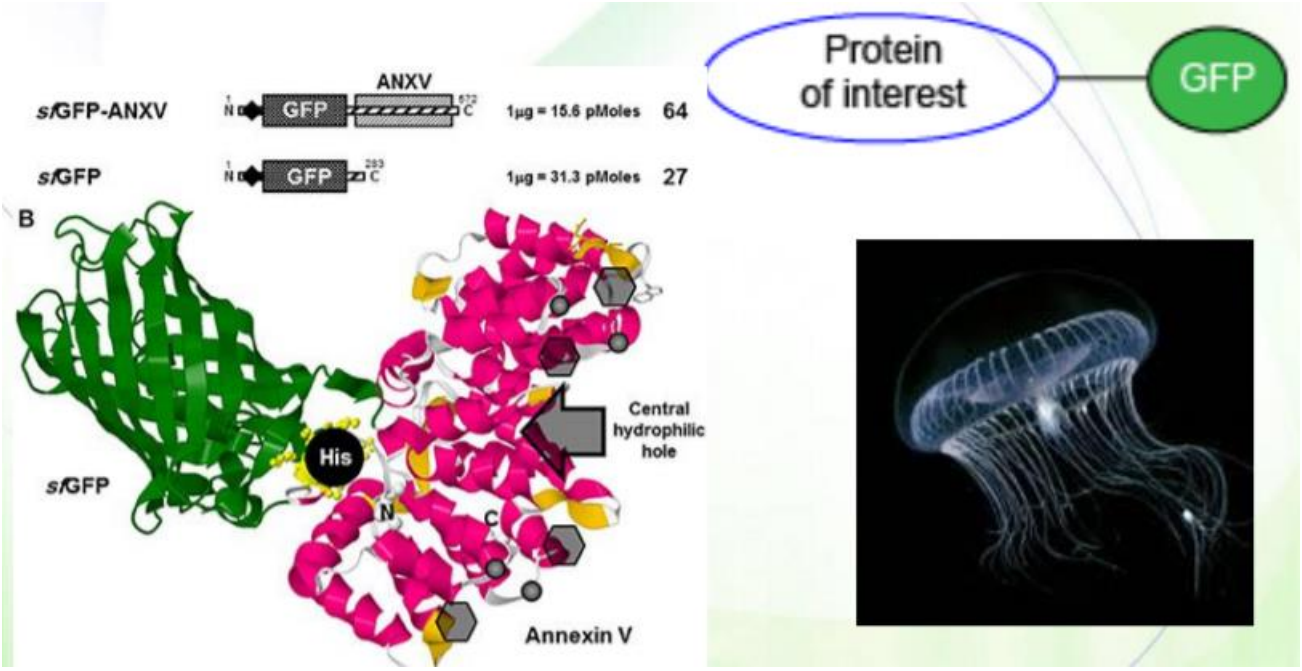
Important: GFP allows for protein detection rather than for purification purposes.

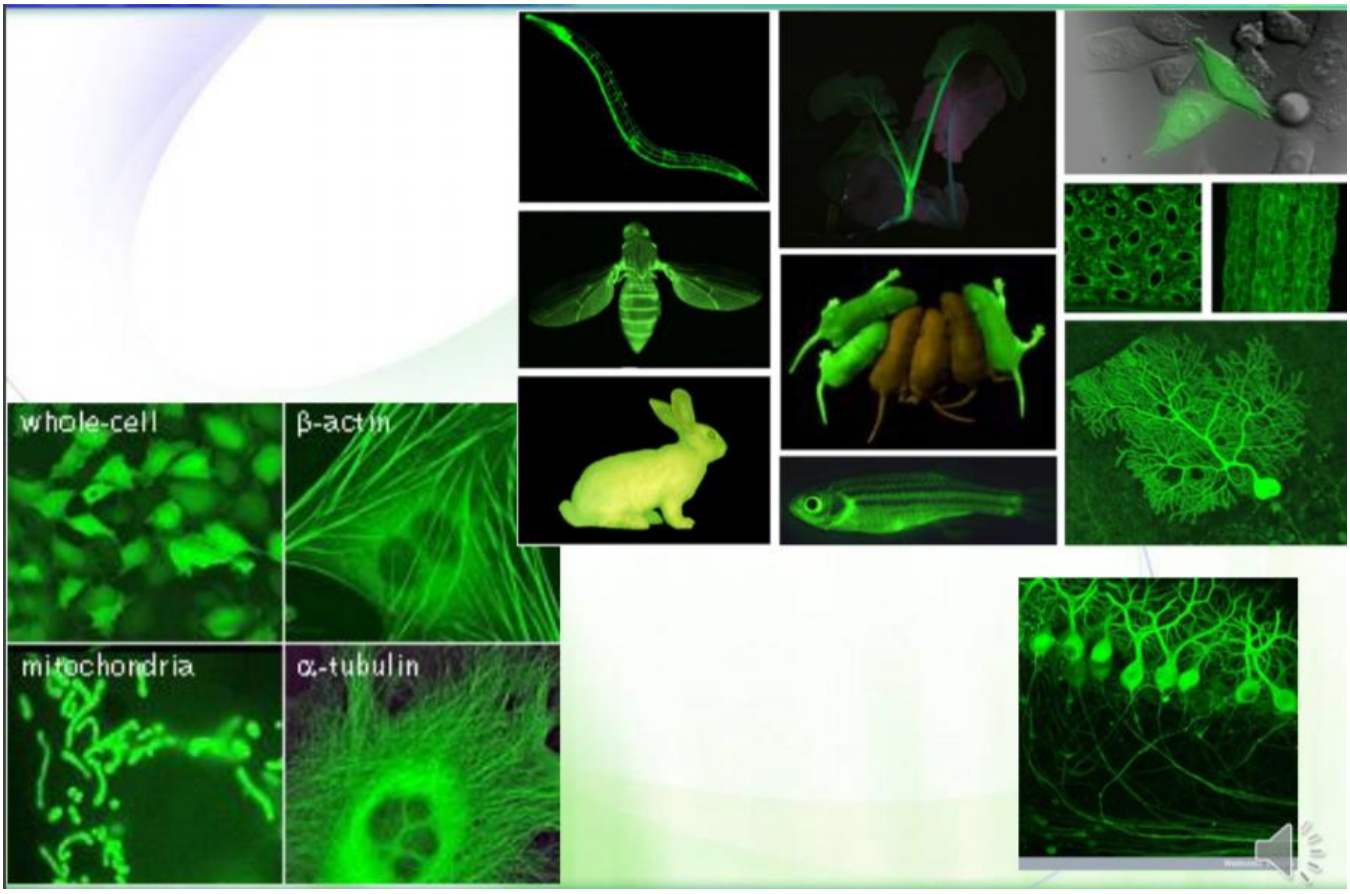
(GFP is a fluorescent protein which gives a green color, so GFP-tagged proteins would

obtain a green color which helps in labelling and detection. Examples of proteins that can be labelled with GFP: actin, tubulin, mitochondrial protein.

Whole cells can also be labelled (like neurons, which allows us to see how they are connected to each other and study neural networks)

Whole animals can be labelled as well (their health is not harmed by the process)





SHORT QUIZ

TEST BANK

1. Which of these bonds the DNA ligase form between two nucleotides of sticky ends?

A) Hydrogen bonds B) Disulfide bond C) Phosphodiester bonds D) Aand C

2. ..... Is a fluorescent protein which gives a ..... color

A) GFP/ Green B) GTP/ Green C) GFP/ Blue D) GTP/ Blue

3. Petri dish are used in .....

A) purification B) Recombinant DNA technology C) DNA Cloning D) B and C

4. One of the following is NOT a challenge that appears when producing human protein in bacteria:

A) Misfolding B) Degradation C) No post-translational modification D) None of the answers are correct

5. The restriction endonuclease (EcoRI) cuts the DNA CTTAAG between:

A) C And T B) Aand A C) T and T D) Aand G.

ANSWERS

Q1	Q2	Q3	Q4	Q5
C	A	C	D	D