Biochemistry and molecular biology

Summer 2019-2020

Course Number: 0501118

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Syllabus

- Molecular techniques
- Introduction into biochemistry
- Acids, bases, pH, and buffers
- Carbohydrates
- Lipids
- Amino acids
- Polypeptides and protein structure
- Protein structure-function relationship (part I: fibrous proteins)
- Protein structure-function relationship (part II: globular proteins)
- Protein structure-function relationship (part III: immunoglobulins)
- Enzymes (introduction)
- Enzymes (kinetics)
- Enzymes (mechanisms and regulation)
- Enzymes (cofactors)
- Biochemical techniques

Recombinant DNA-based molecular techniques

Prof. Mamoun Ahram
Summer 2020

Restriction endonucleases

- Endonucleass are ezymes that degrade DNA within the molecule.
- Restriction endonucleases: Bacterial enzymes that recognize and cut (break) the phosphodiester bond between nucleotides at *specific* sequences (4- to 8-bp restriction sites) generating restriction fragments.

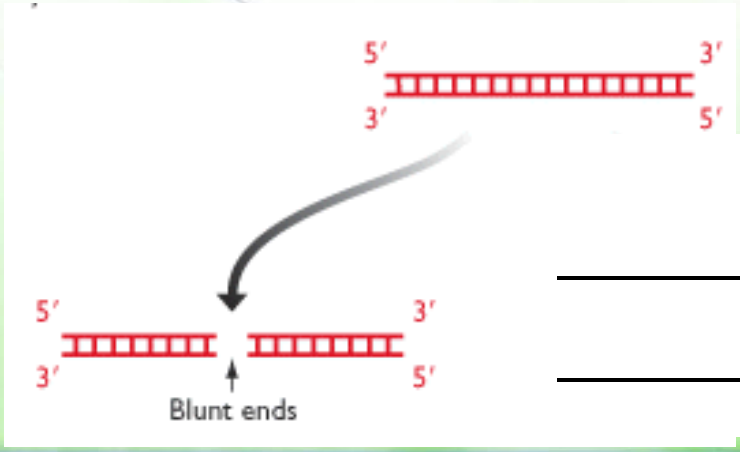
Palindromic sequences

- 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).

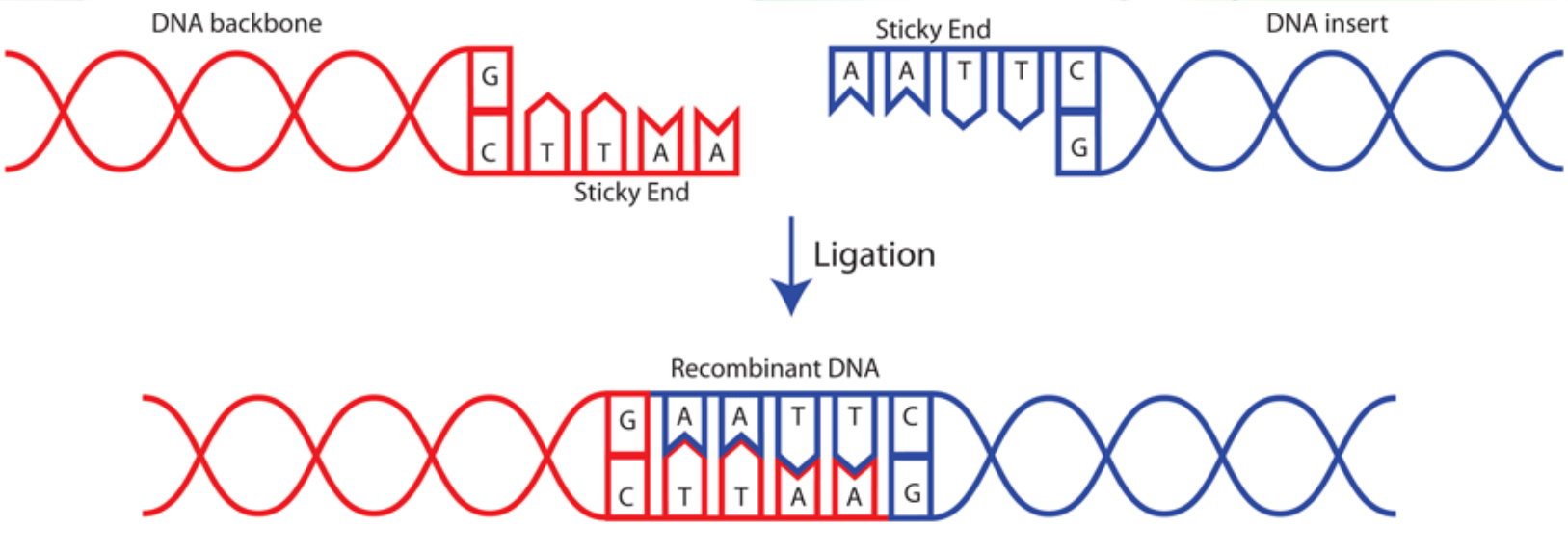
```
EcoRI       5'  GAATTC  3'
            3'  CTTAAG  5'

HindIII     5'  AAGCTT  3'
            3'  TTCGAA  5'

SmaI        5'  CCCGGG  3'
            3'  GGGCCC  5'
```

Types of cuts by restriction endonucleases

- Restriction enzymes cut DNA in two different ways:
  - Blunt: enzymes cut at the same position on both strands giving a blunt ended fragments.
  - Staggered (off-center): enzymes cut the two DNA strands at different positions generating sticky or cohesive ends.
    - The DNA fragments have short single-stranded overhangs at each end.

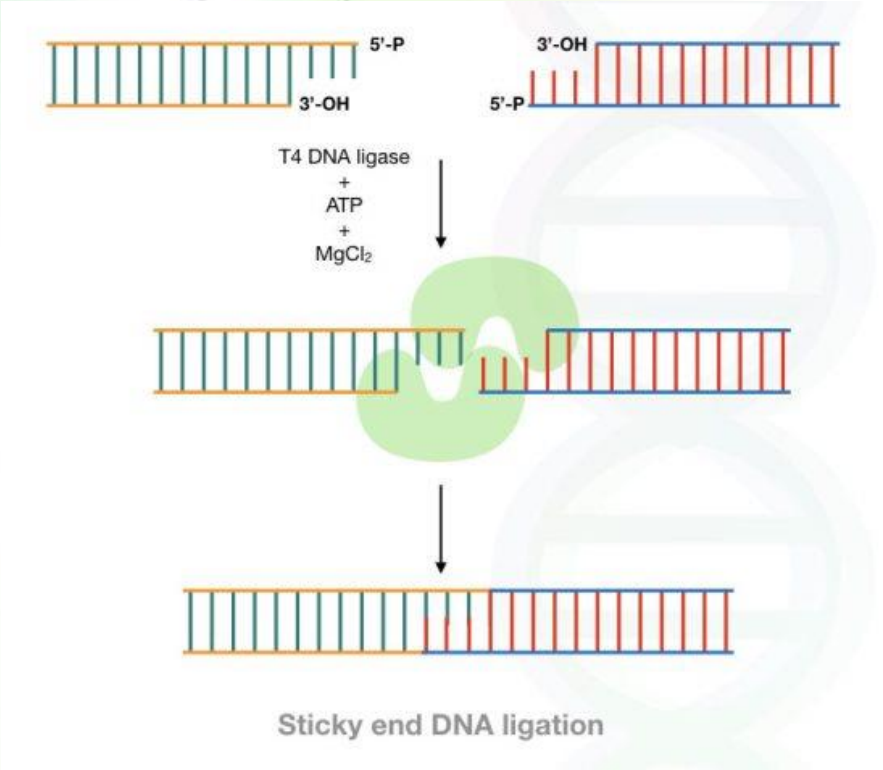


Zoom into the sticky ends



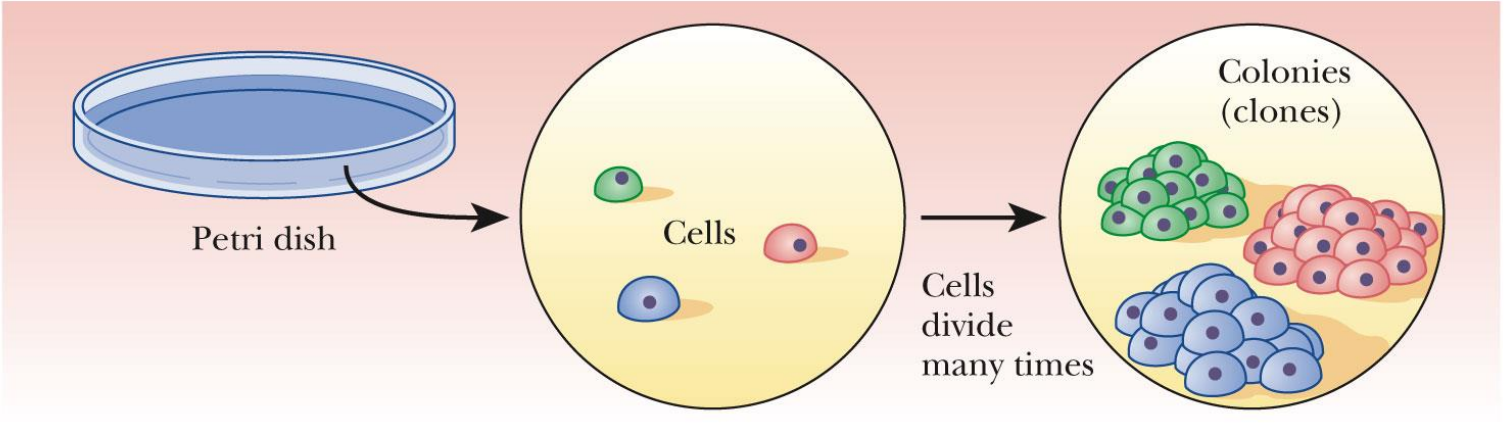
DNA ligase

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



Cloning

- Cloning means that you make several copies of one thing.
- A clone is a 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.



How do we clone a DNA molecule?

- a DNA fragment of interest is inserted into a DNA carrier (called a vector) that can be replicated.
- The resulting DNA molecule is what is known as a recombinant DNA molecule.

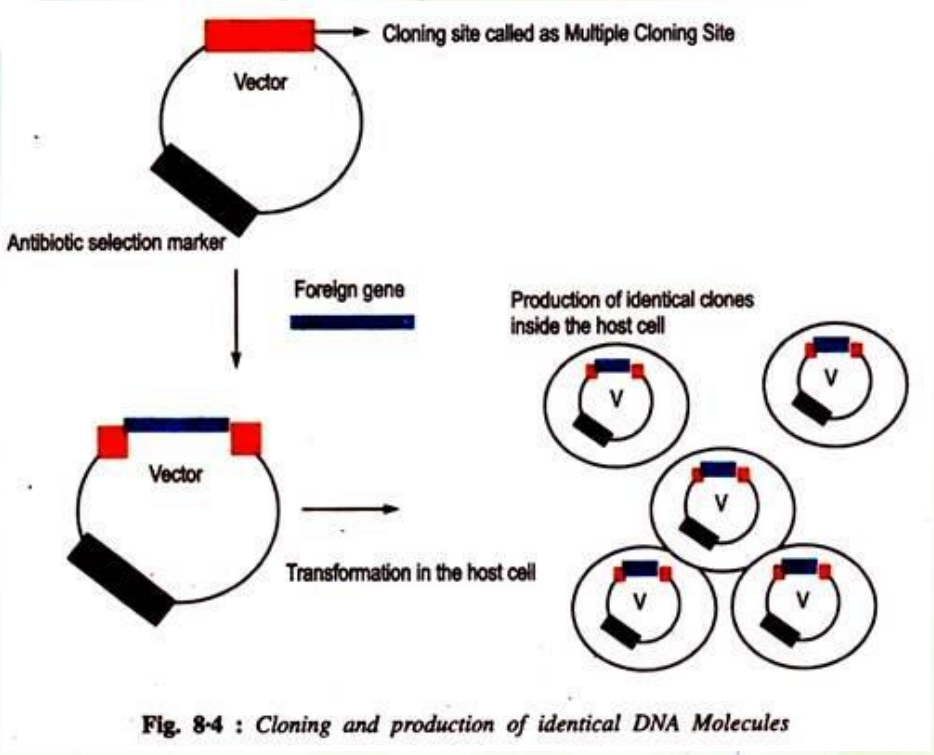
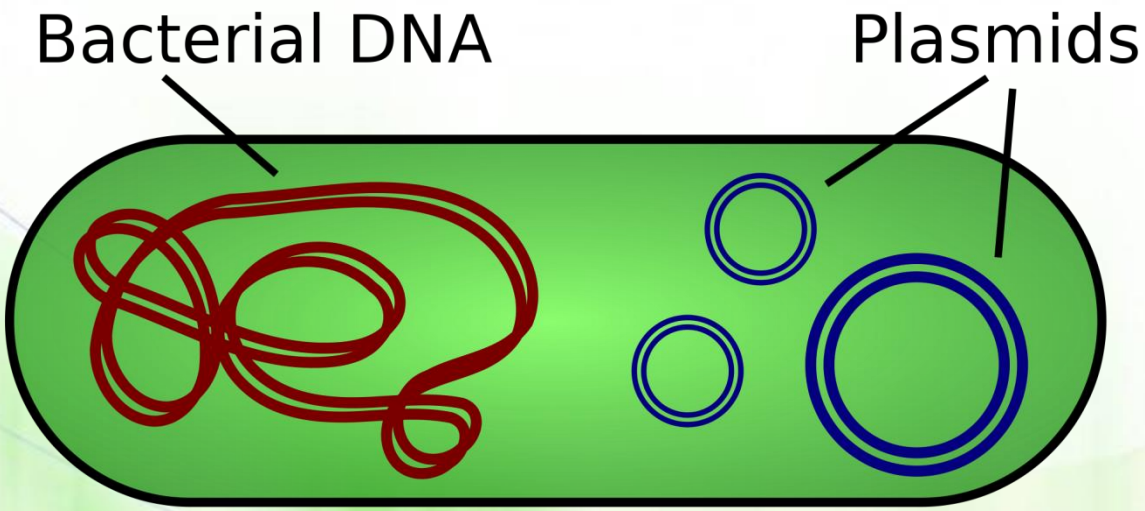


Fig. 8-4 : *Cloning and production of identical DNA Molecules*

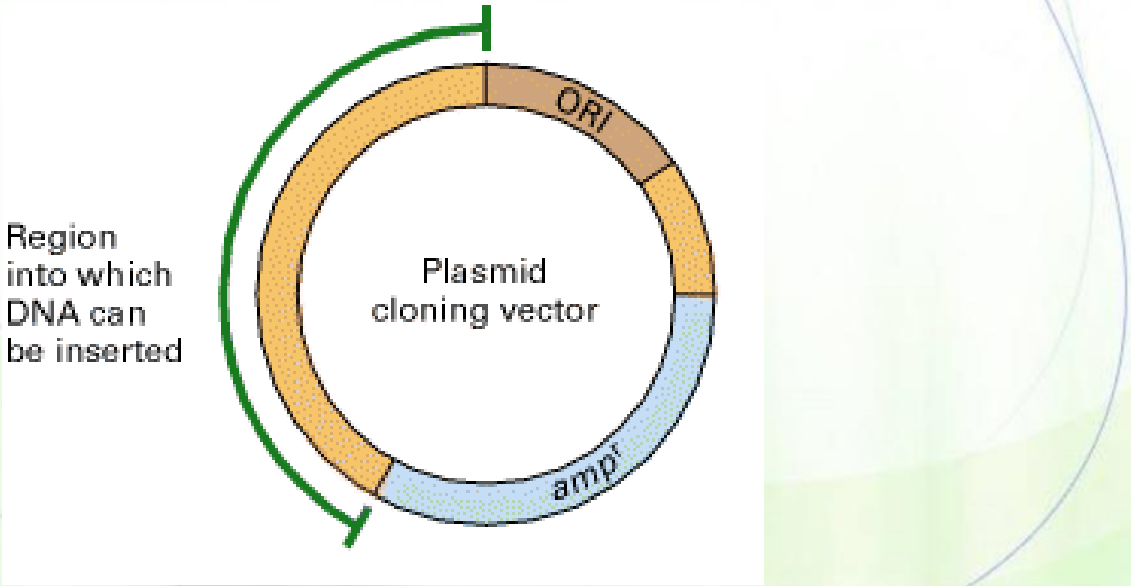
Using plasmids as vectors

- Bacterial plasmids are considered excellent vectors.
- These are bacterial circular DNA that is not part of the main circular DNA chromosome of the bacterium.
- A plasmid exists as a closed circle and replicates independently of the main bacterial genome.



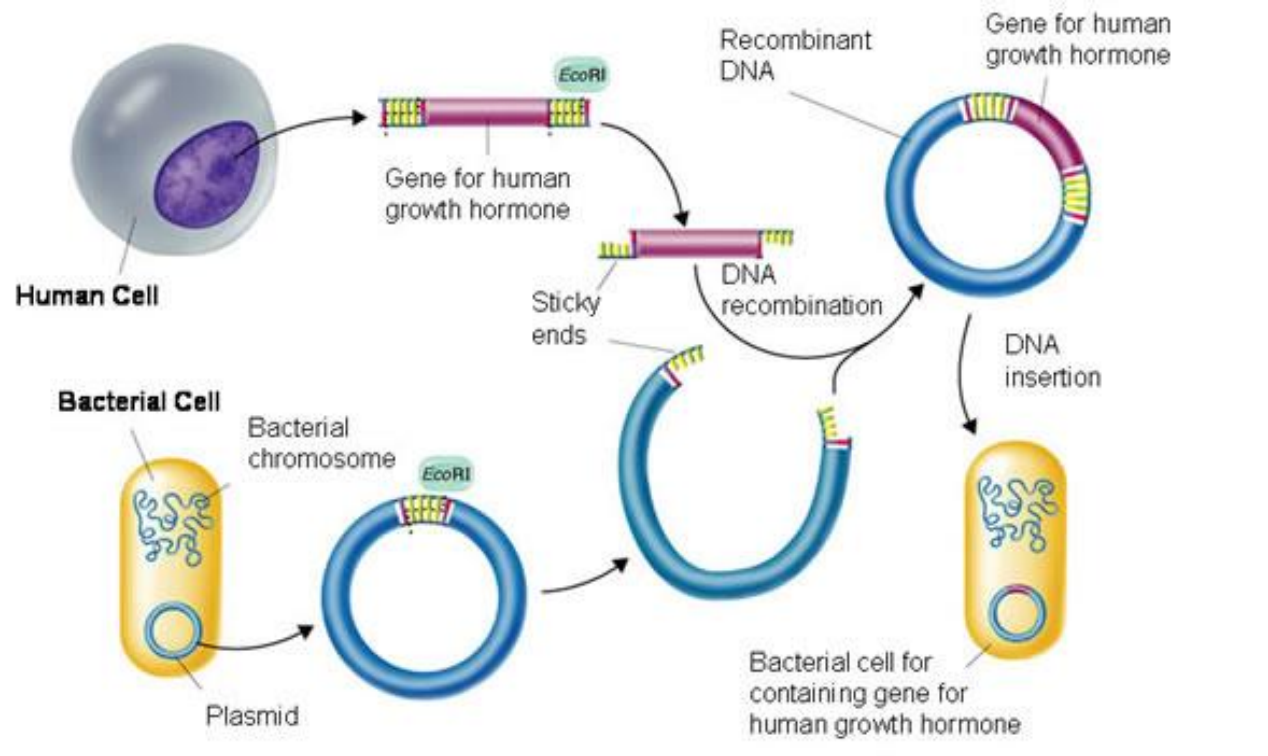
Features of plasmids

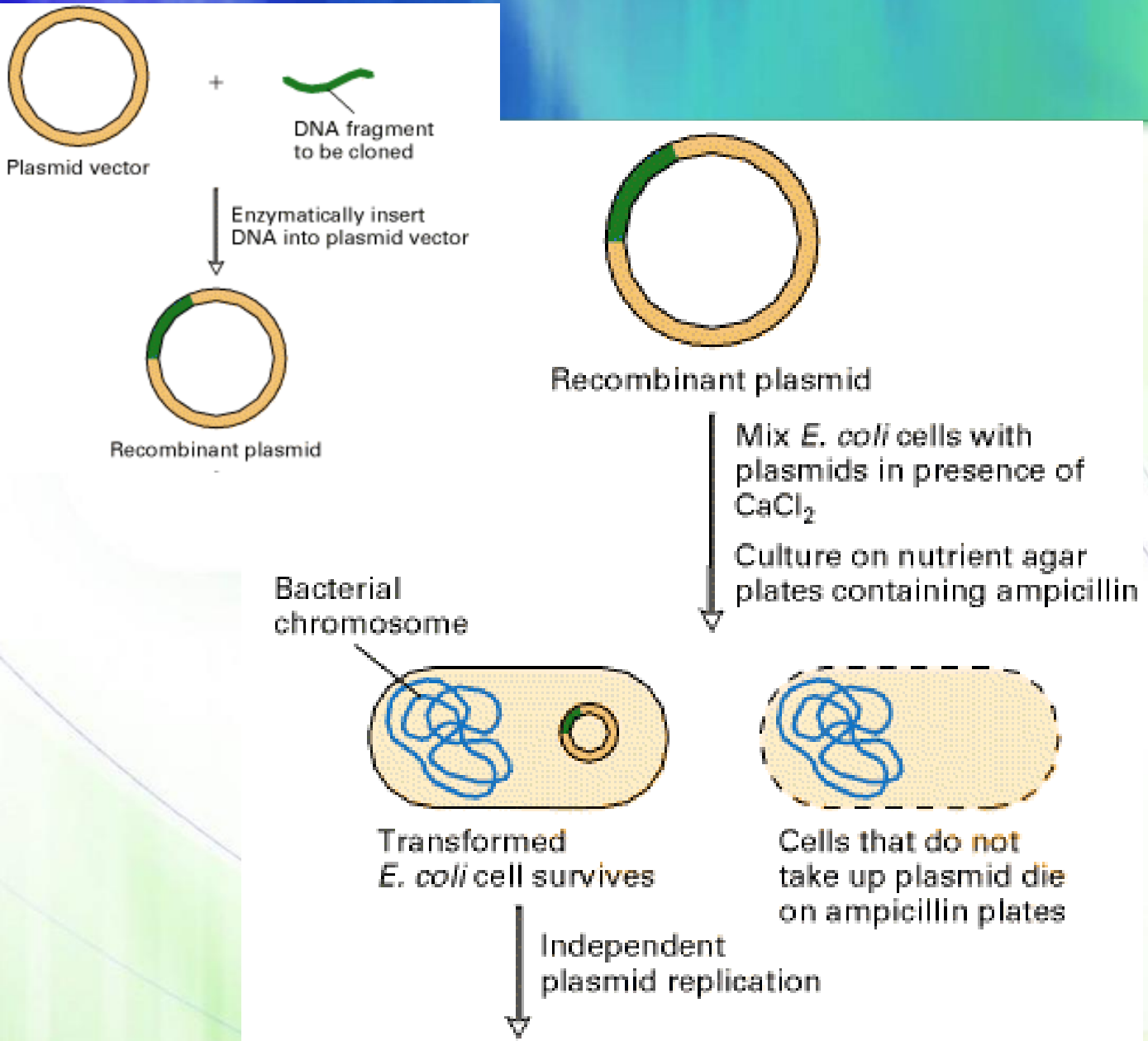
- Most plasmid vectors contain at least three essential parts required for DNA cloning:
  - Can replicate
  - Can be selected for/against by an internal drug-resistance gene (selectable marker)
  - Can insert a foreign DNA fragment

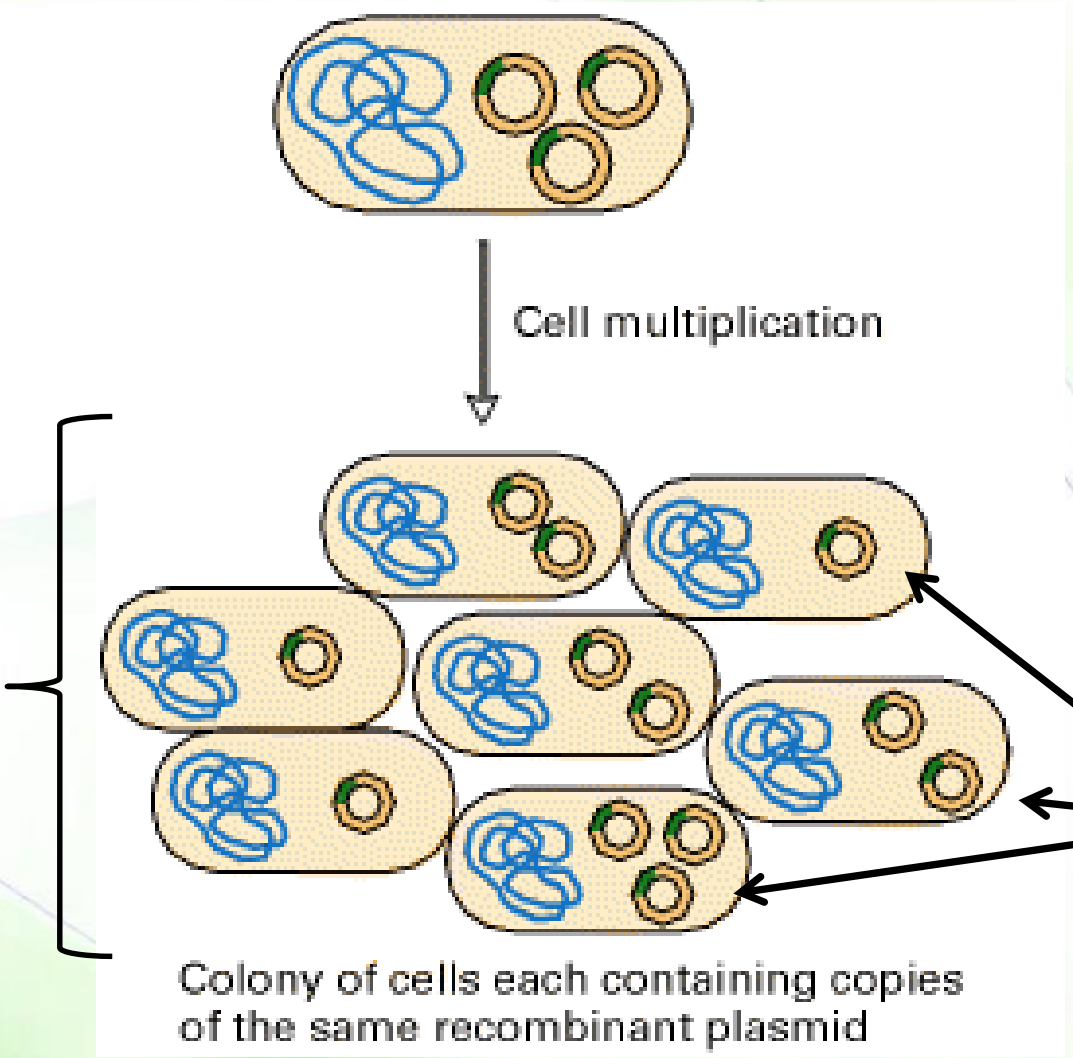


Making of recombinant DNA

- Both DNA fragments (the DNA to be cloned and a vector) are cut by the same restriction endonuclease that makes DNA fragments with same sticky-ends that hybdridize to each other, when mixed.



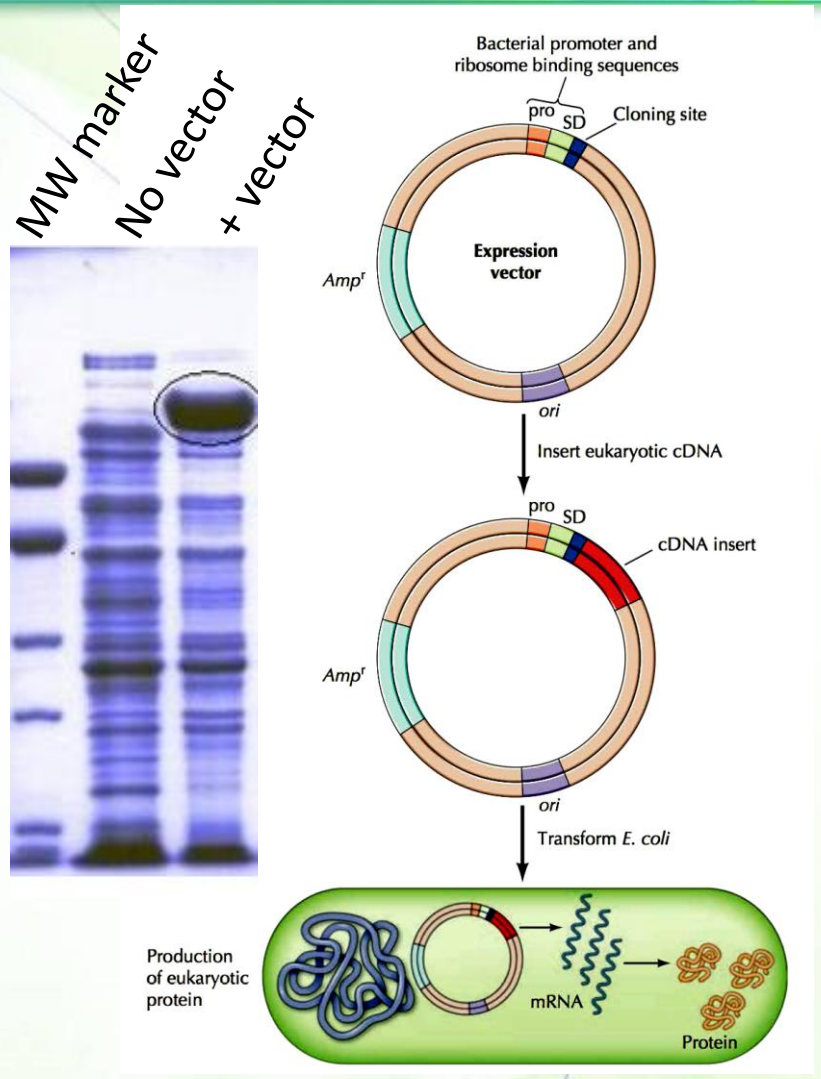

Plasmid vector
+
DNA fragment to be cloned
Enzymatically insert DNA into plasmid vector
Recombinant plasmid
Recombinant plasmid
Mix E. coli cells with plasmids in presence of $CaCl_2$
Culture on nutrient agar plates containing ampicillin
Bacterial chromosome
Transformed E. coli cell survives
Cells that do not take up plasmid die on ampicillin plates
Independent plasmid replication


Cell multiplication
Cell clones
Cloned DNA
Colony of cells each containing copies of the same recombinant plasmid

Expression vectors

- Expression vectors contain additional sequences:
  - Promoter sequences upstream of gene to be inserted
  - Ribosomal binding sequences (Shine-Dalgarno [SO] sequences)
  - Transcription termination sequence

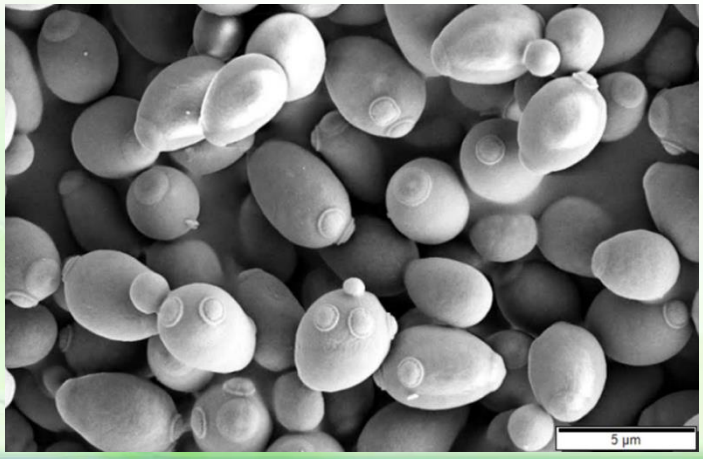
- The protein is expressed and purified.
- *Examples: insulin, growth hormone, plasminogen activator, erythropoietin*



Challenges

- Internal disulfide bonds in bacteria
- No post-translational modification (example: glycosylation)
- Misfolding
- Degradation

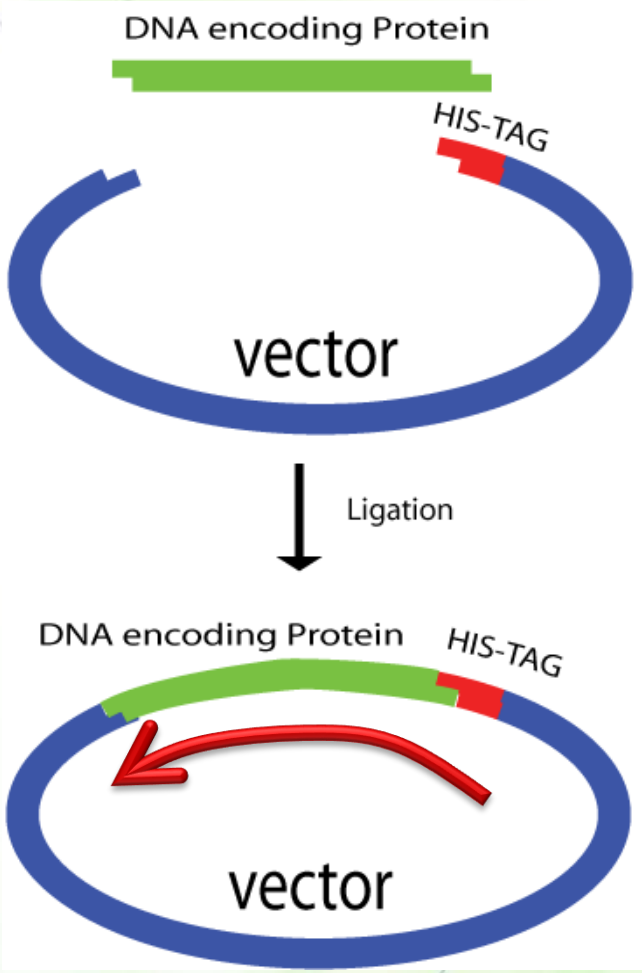
- Solution: use a eukaryotic system such as yeast



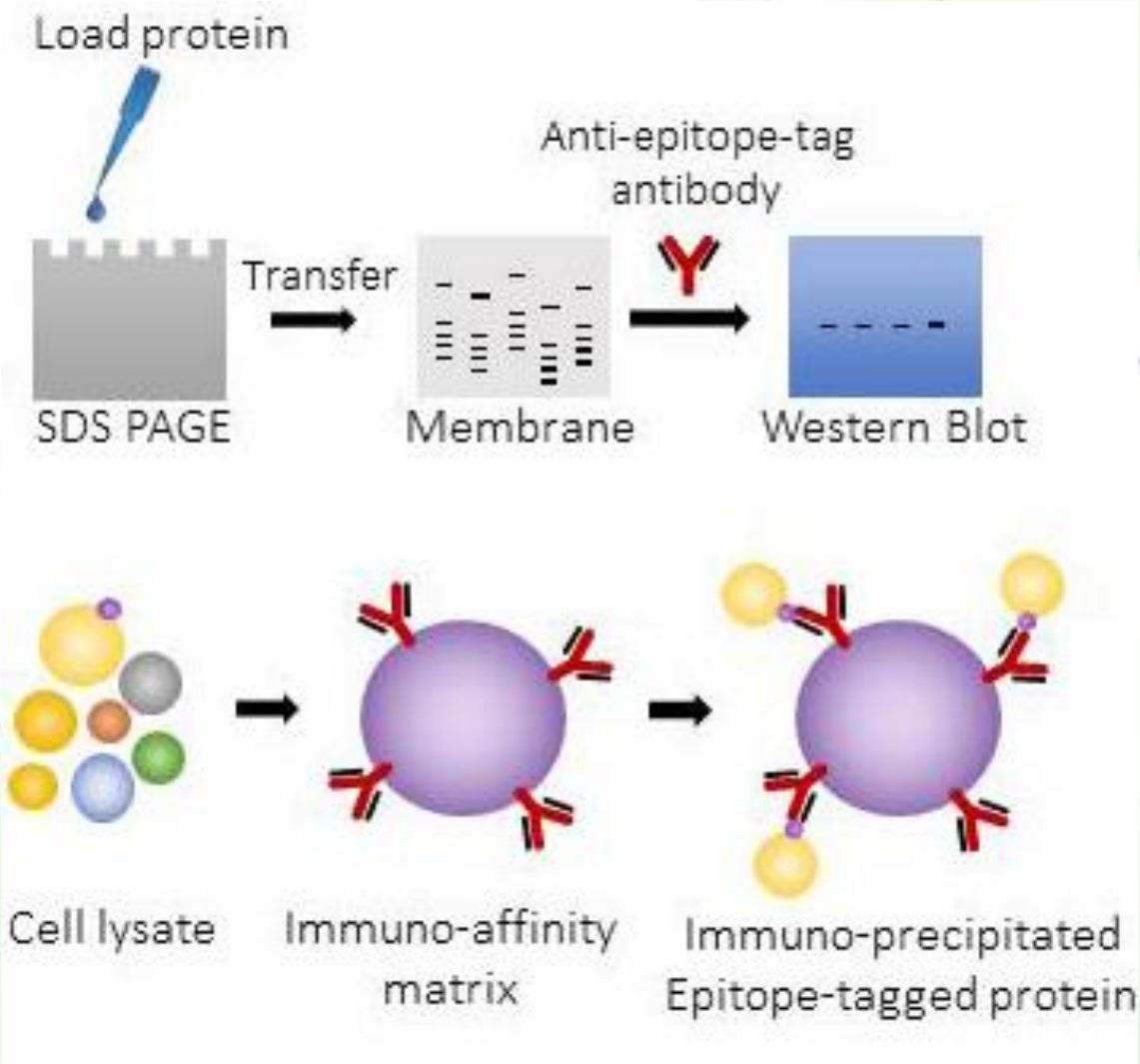
Protein tagging or creation of protein hybrids

Proteins can be "tagged"

- A protein-encoding gene is cloned in a special vector containing a tag gene producing a protein with an extra sequence of amino acids called tags.
- These tags allow easy protein purification and detection.



Uses of protein tags

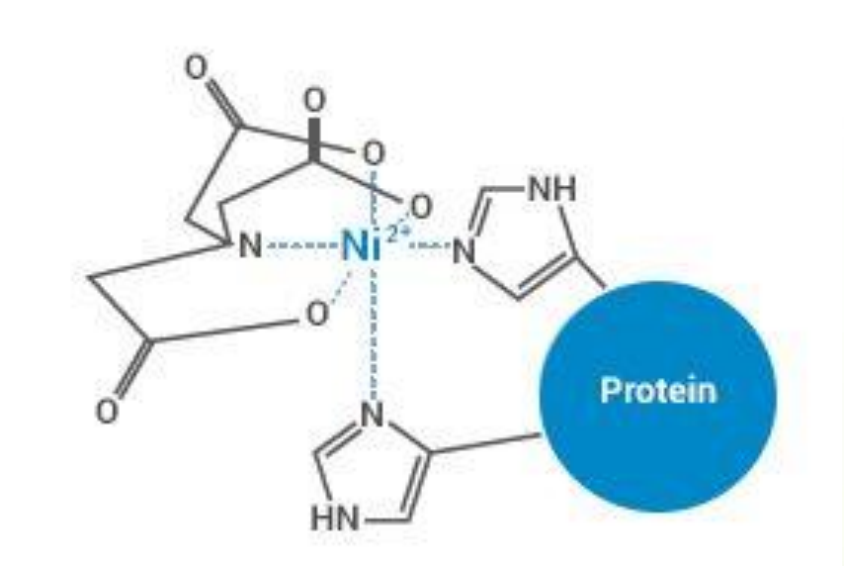


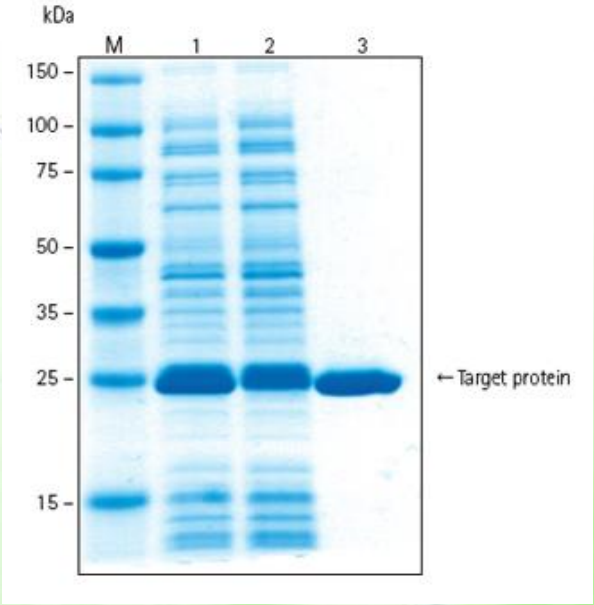
Major protein and epitope tags

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

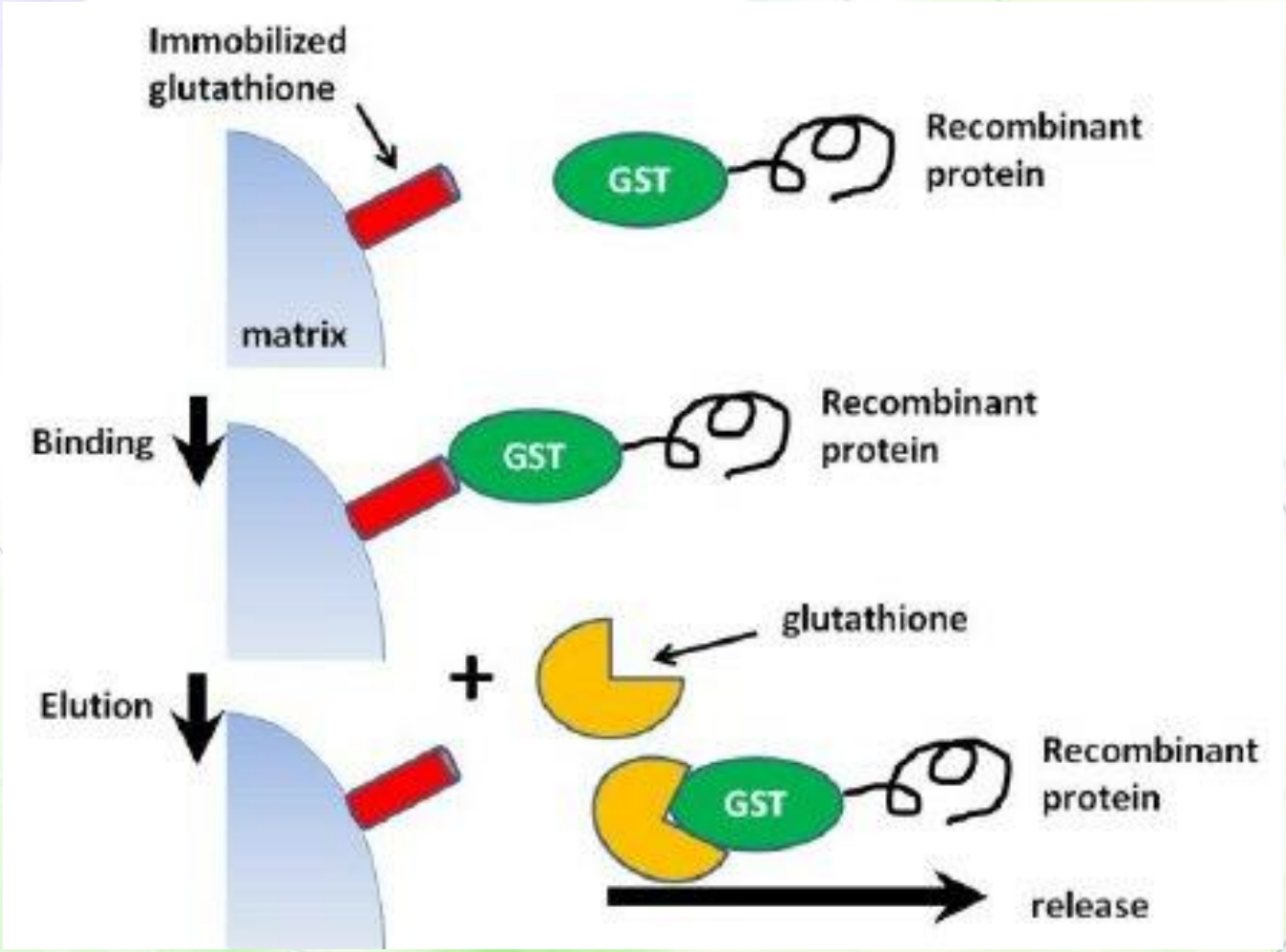
His tag

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

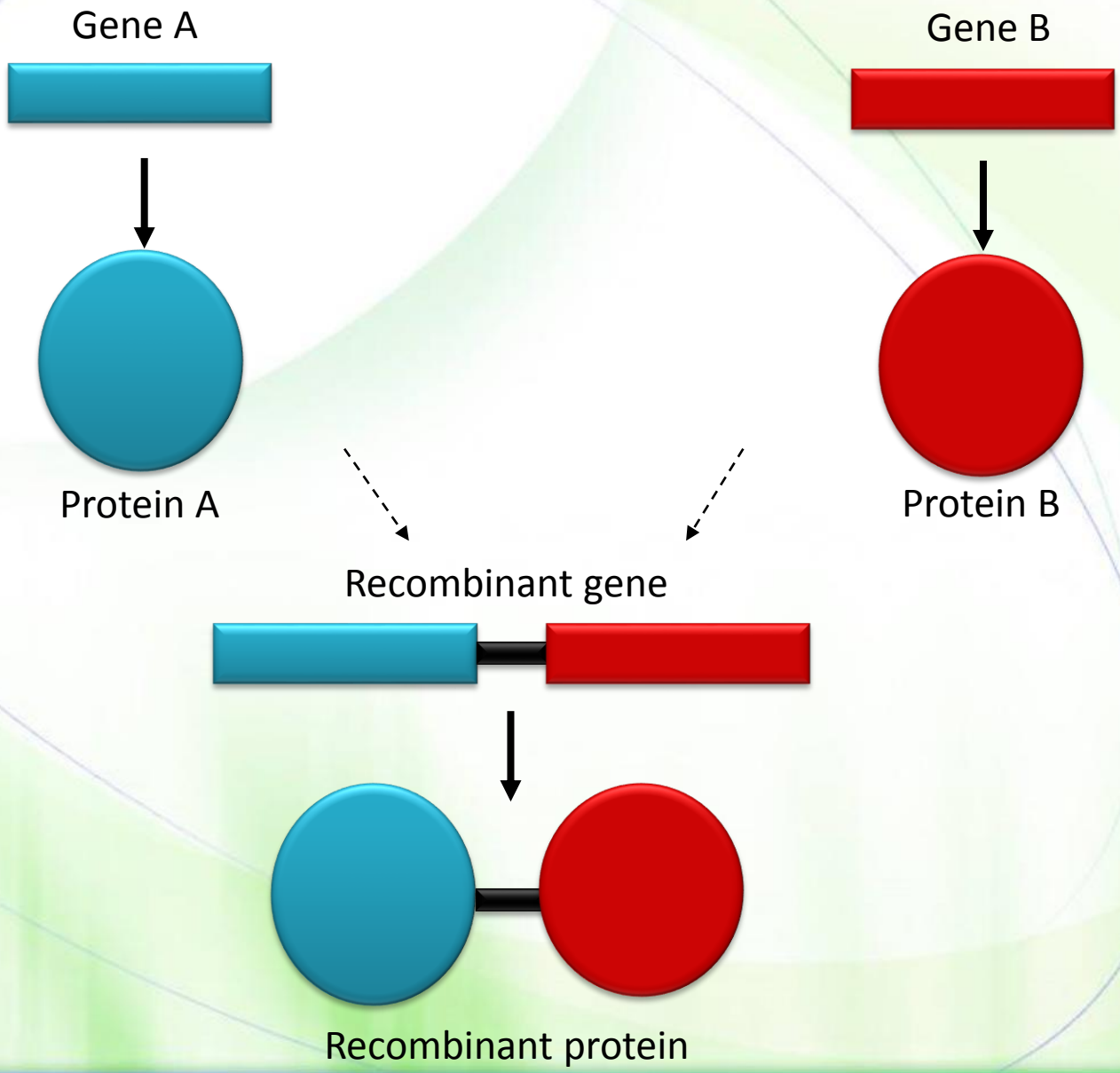




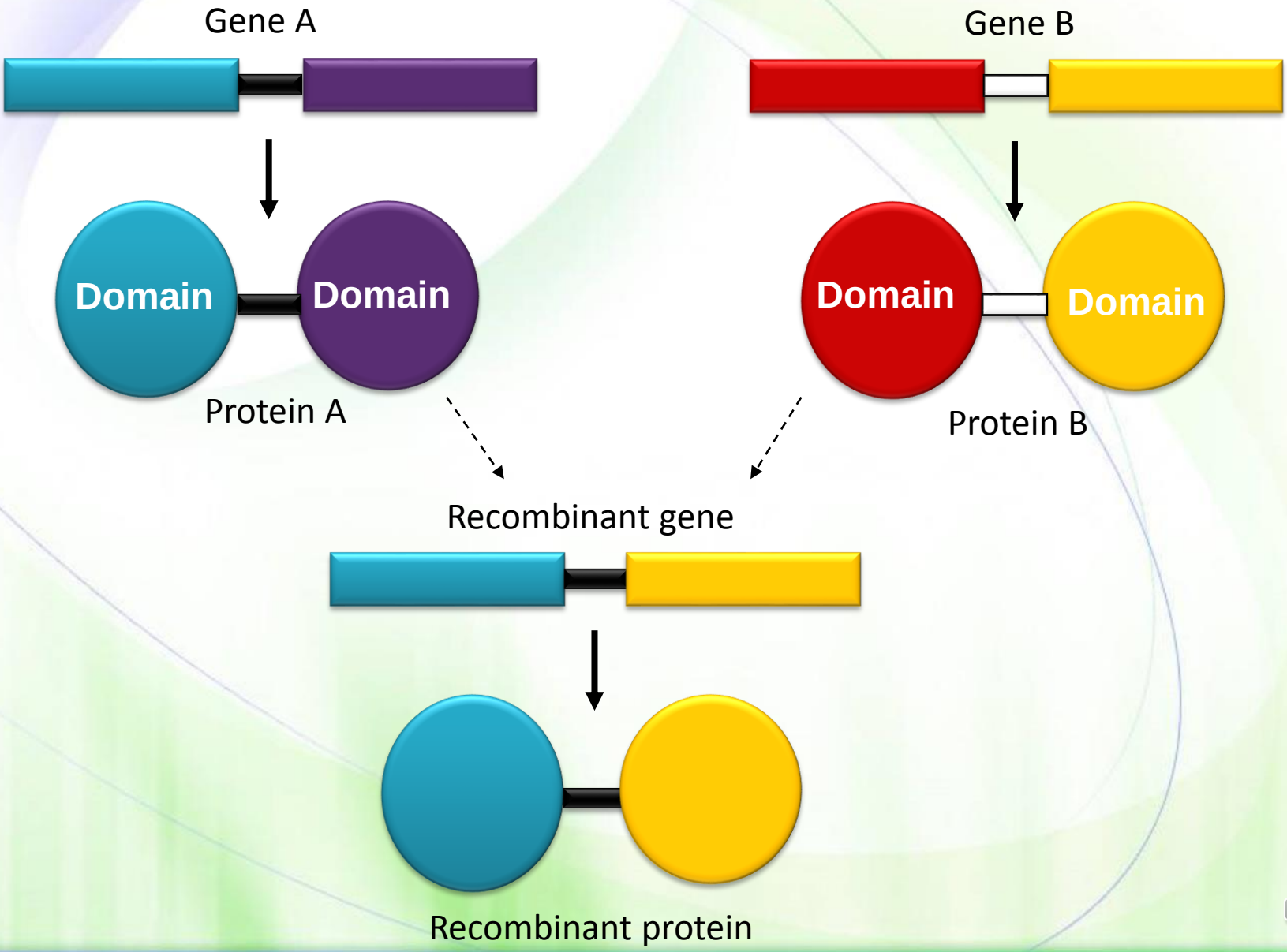
Purification of GST-tagged proteins



Production of a recombinant protein



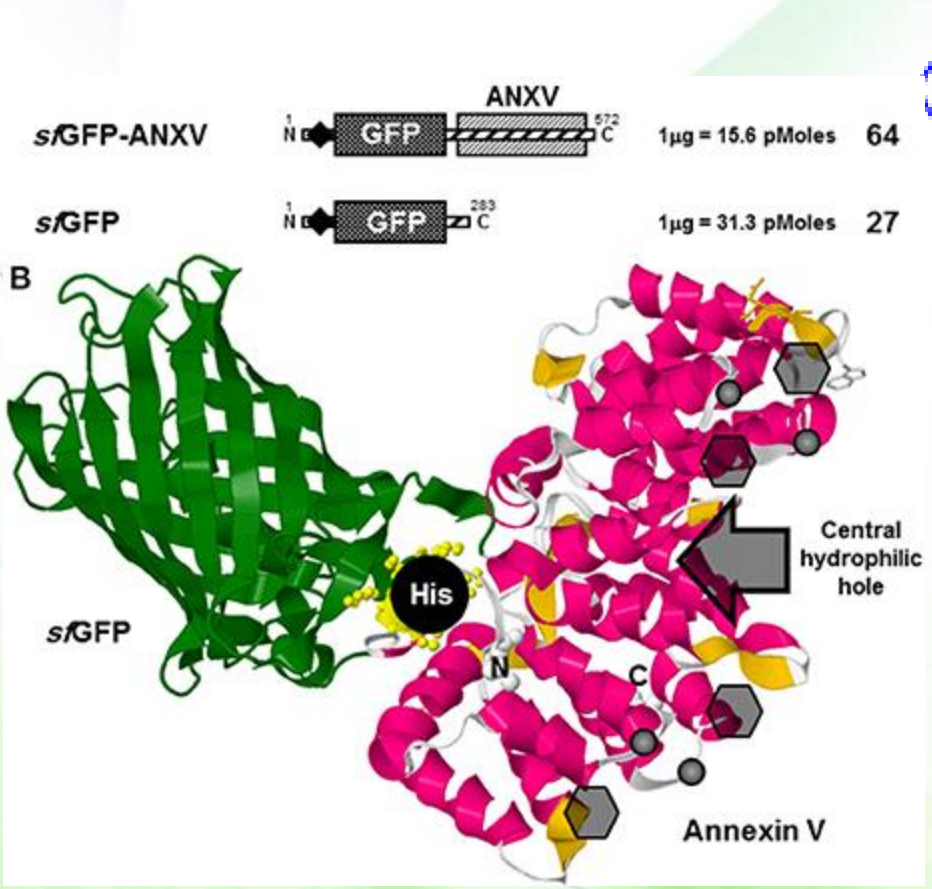
Production of a recombinant protein



GFP-tagged proteins

The power of domains

- GFP allows for protein detection rather than for purification purposes.



A world of possibilities