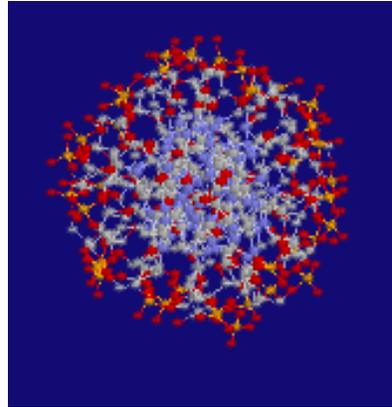


# **Week 8. Gene Cloning**

# Gene Cloning



**Department of Microbiology**

# Gene Cloning

- What is cloning?
- What is gene cloning? How does it differ from cloning a whole organism?
- Why do we clone genes?
- How do we clone genes?
- What is DNA library and cDNA library?

# RESTRICTION ENZYMES

They are bacterial proteins (endonucleases) cutting DNA molecules from specific restriction sites.

- **restriction site:** These are the 4-8 based specific DNA sequences those recognized by a restriction enzyme
- **restriction fragment:** Smaller DNA fragment separated from a larger DNA fragment following a digestion with one or more restriction enzymes
- hundreds of different RE are present, each has a specific restriction site

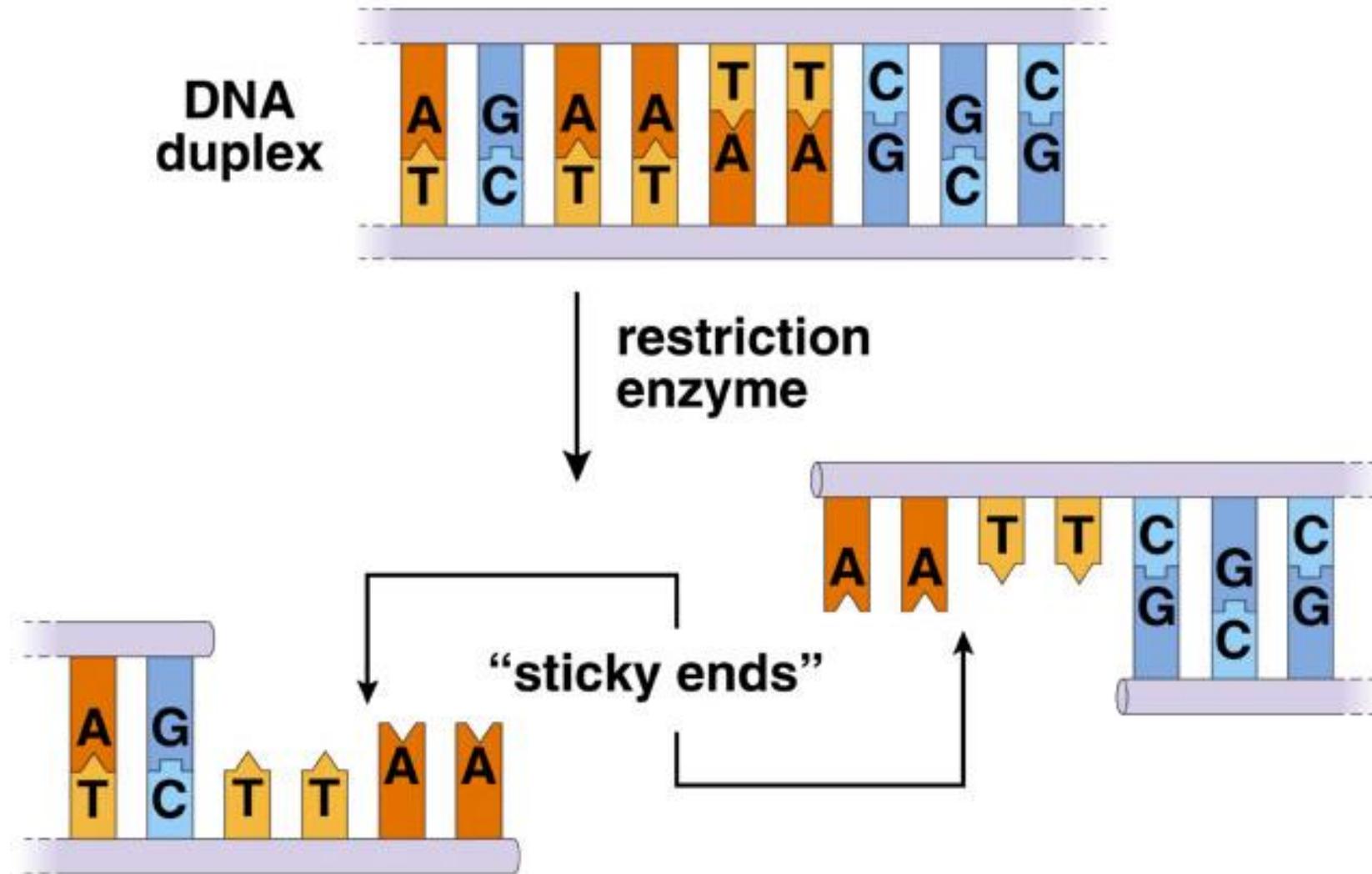
## Nomenclature and Description of REs (EcoRI)

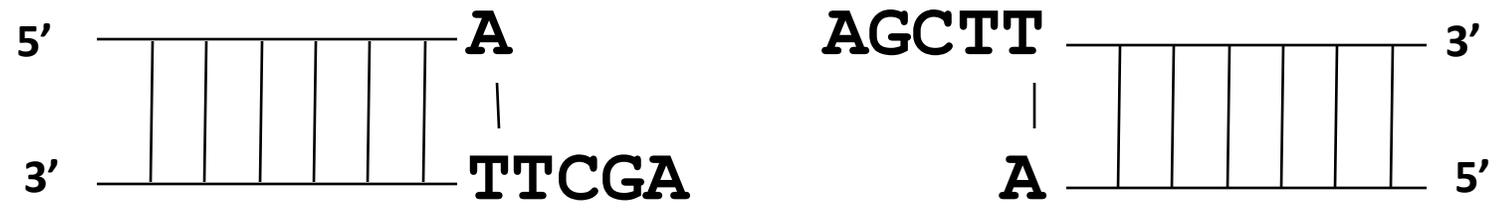
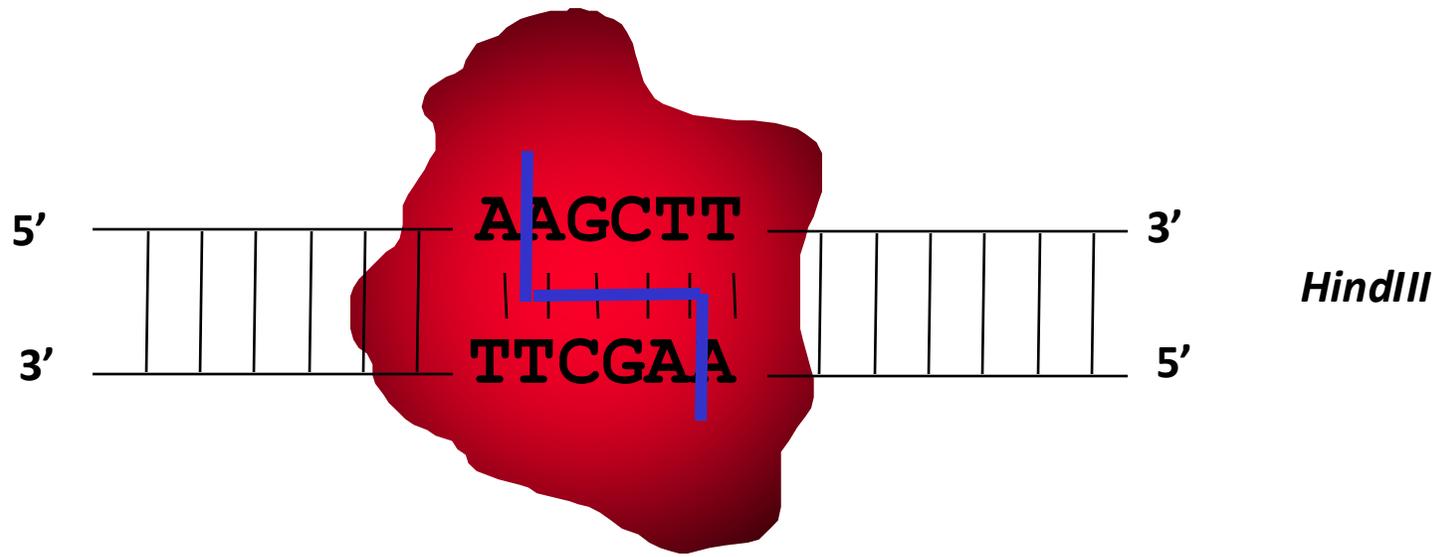
Abbreviation	Meaning	Definition
<b>E</b>	<i>Escherichia</i>	genus
<b>co</b>	<i>coli</i>	species
<b>R</b>	RY13	strain
<b>I</b>	First identified	Identification priority

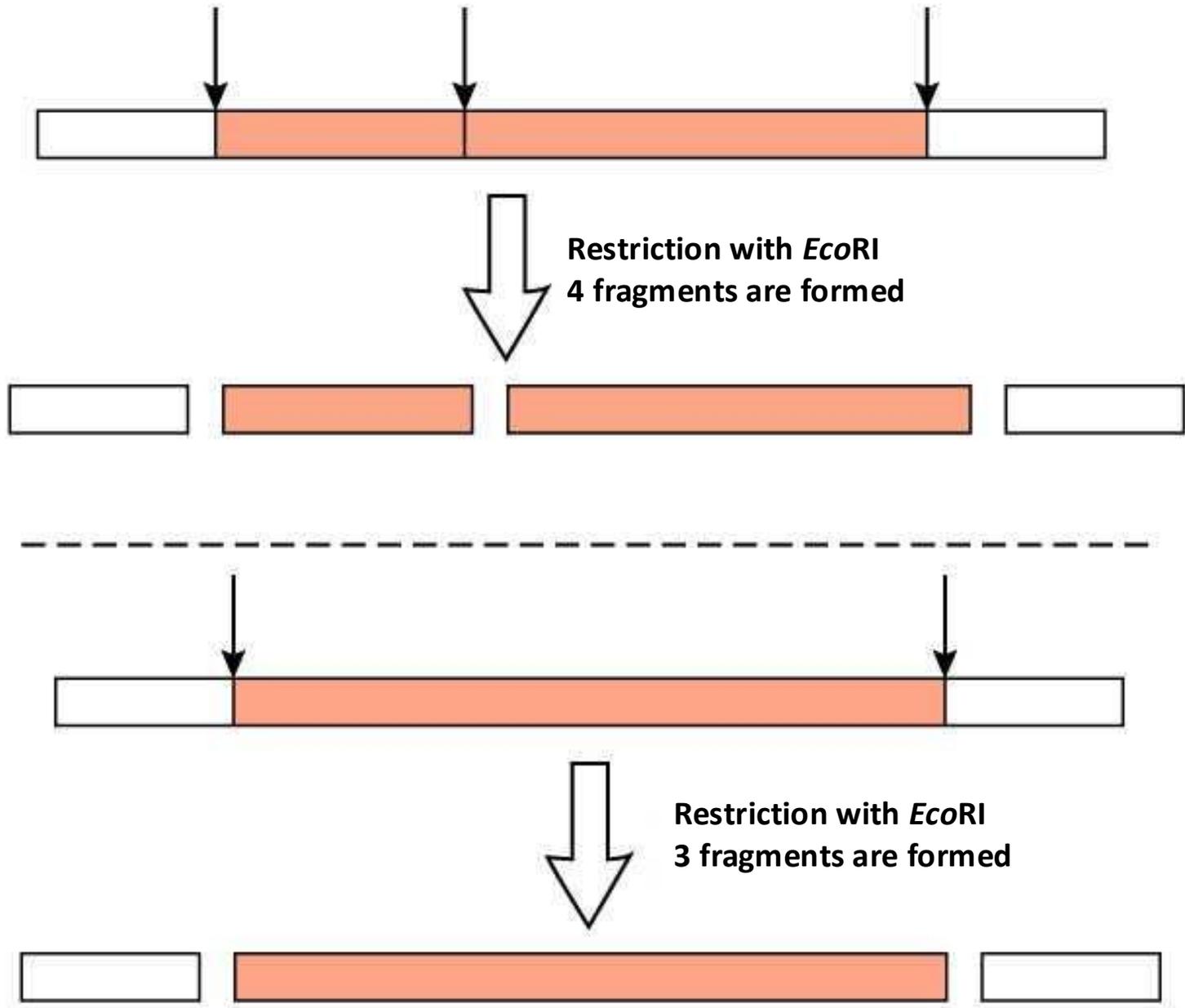
**Table. Specific restriction sites of some of the REs**

<b>Organism</b>	<b>RE Name</b>	<b>Restriction site</b>
<i>Escherichia coli</i>	<i>EcoRI</i>	G <u>A</u> ATTC
<i>Escherichia coli</i>	<i>EcoRII</i>	<u>C</u> CCAGG
<i>Haemophilus influenzae</i>	<i>HindII</i>	GTPPy <u>P</u> uAC <u>C</u>
<i>Haemophilus hemolyticus</i>	<i>HhaI</i>	G <u>C</u> G <u>C</u>
<i>Bacillus subtilis</i>	<i>BsuRI</i>	G <u>C</u> C
<i>Brevibacterium albidum</i>	<i>Ball</i>	TGG <u>C</u> CA
<i>Thermus aquaticus</i>	<i>TaqI</i>	T <u>C</u> GA

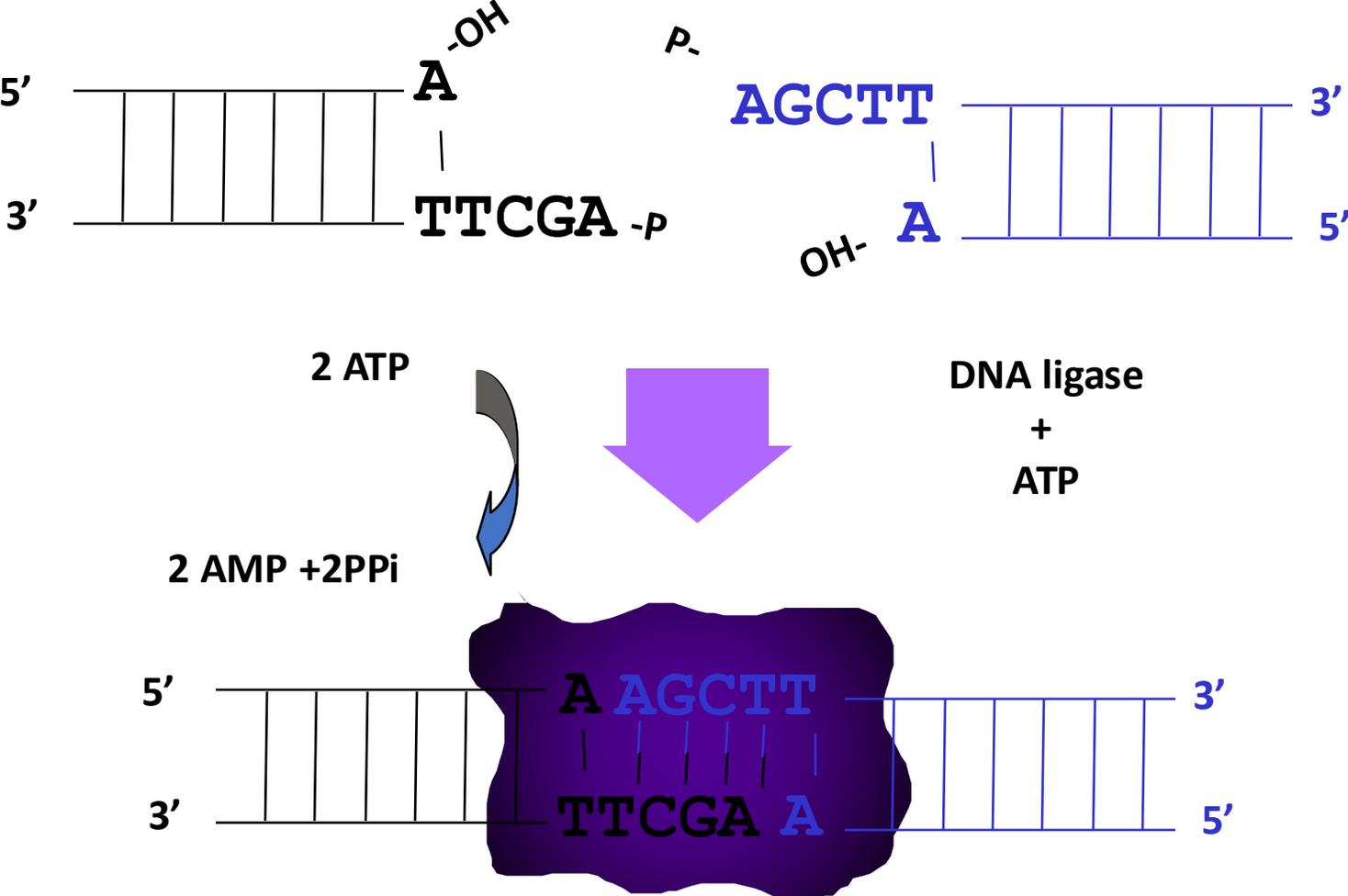
# RE sites







# DNA CLONING: DNA LIGATION



# Cloning (Definition)

- Greek - clone, twins
- Isolation and mass production of a specific DNA fragment (clone) out of a complex DNA mixture,
- Asexual production of a content of an individual
- A group containing copy/copies of whole or part of a macromolecule
- Individual genetically similar to her/his parents formed from a single somatic cell of her/his parent (dictionary)

# Stages of Gene Cloning

- Isolation and purification of a gene carrying DNA (or RNA) fragment from a complex DNA molecule,
- Determination of the location of the gene of interest
- Incision of the gene
- Isolation of carrier vector DNA
- Insertion of gene DNA into the vector DNA (Recombination)
- Electroporation and/or transformation of established recombinant vector DNA to recipient cell (procaryotic/eucaryotic)
- Selection
- Control of gene products

Do not forget! All organisms can be cloned but  
in a different way!!!



# Why do we clone DNA?

- Determination of a nucleotide sequences of a specific gene after isolation --- Nowadays better ways for the purpose!!!
- Determination of control DNA sequences
- Investigation of protein/enzyme/RNA functions
- Detection of mutations i.e. Detection of gene defects related to specific diseases
- Production of substances in organisms for specific purposes i.e. Insulin production İnsulin üretimi, establishment of resistant species

# How do we clone DNA?

- DNA is extracted from organic material i.e. blood, tissue, from another bacteria
- REs, i.e. *EcoRI*, *HindIII*, cut DNA into smaller fragments
- Different DNA fragments cut by the same enzyme could be ligated or recombined

Blood sample



DNA



Restriction enzymes

# Materials used in DNA Cloning

**RESTRICTION ENZYMES**

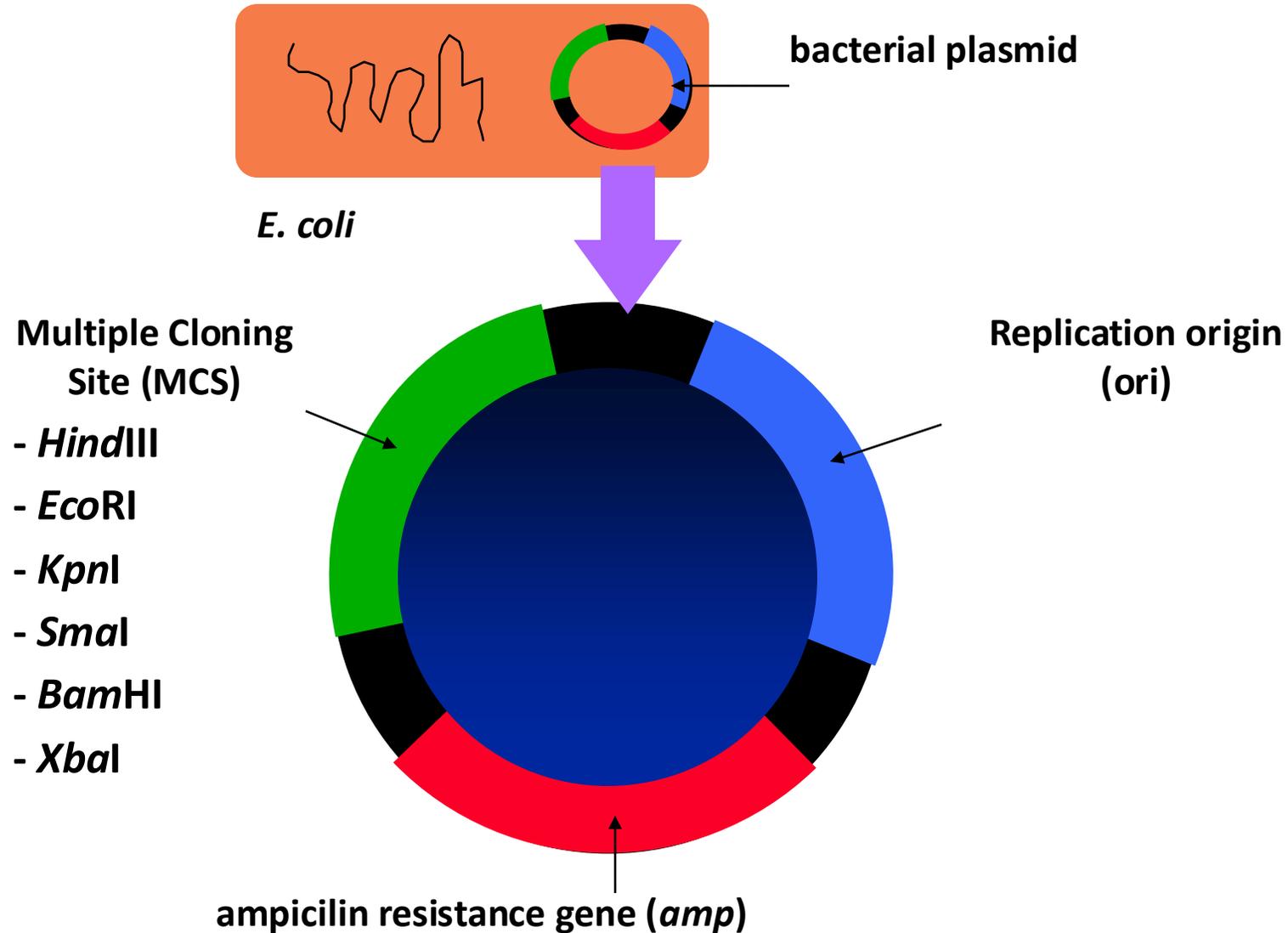
**VECTORS**

**DNA LIGASE ENZYME**

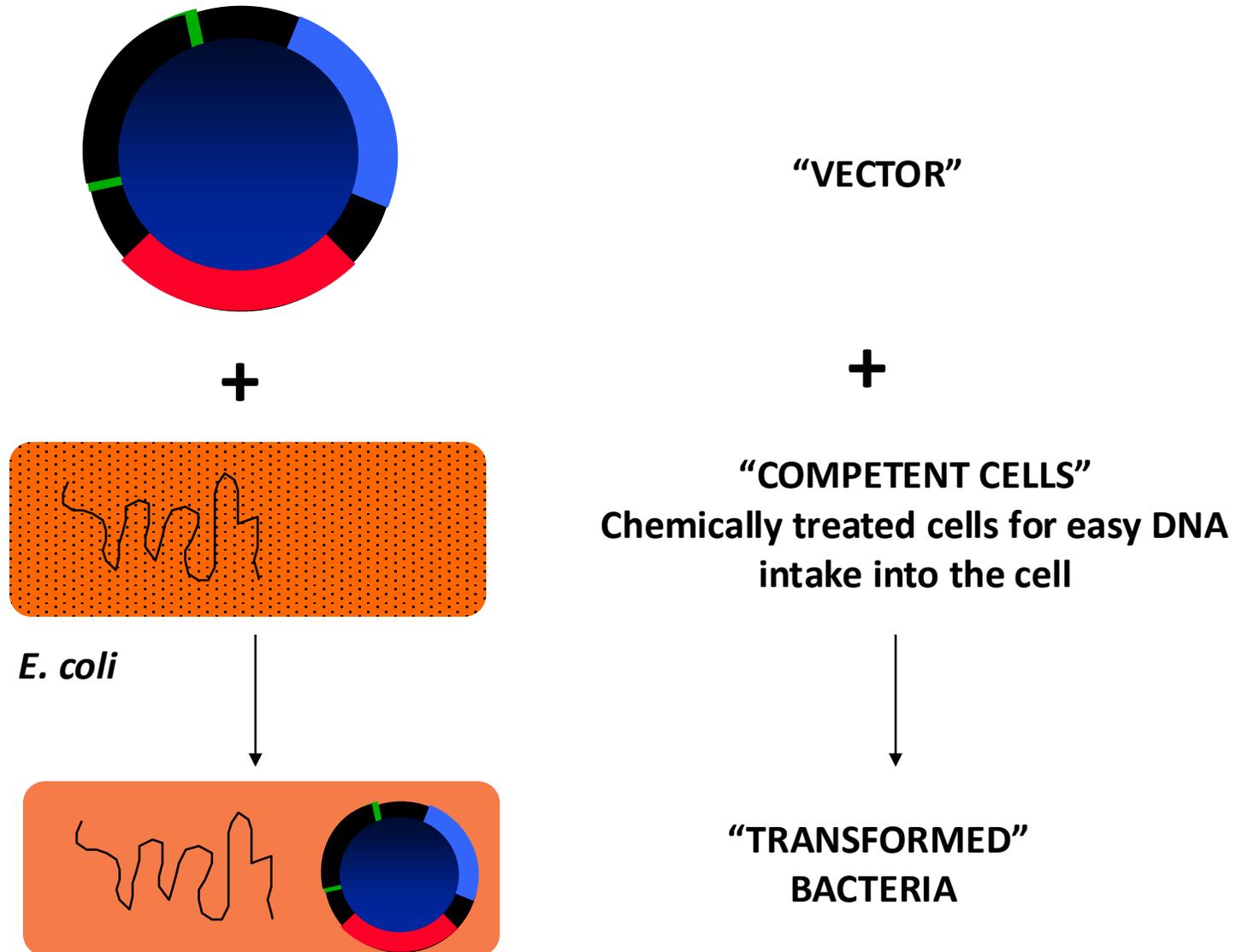
**COMPETENT BACTERIAL CELLS**

**ANTIBIOTICS**

# DNA CLONING: plasmid vectors



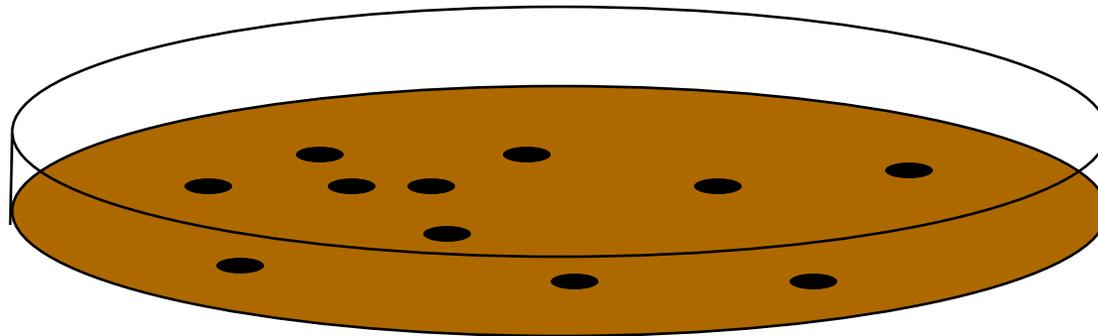
# DNA CLONING: TRANSFORMATION



# DNA CLONING: SELECTION

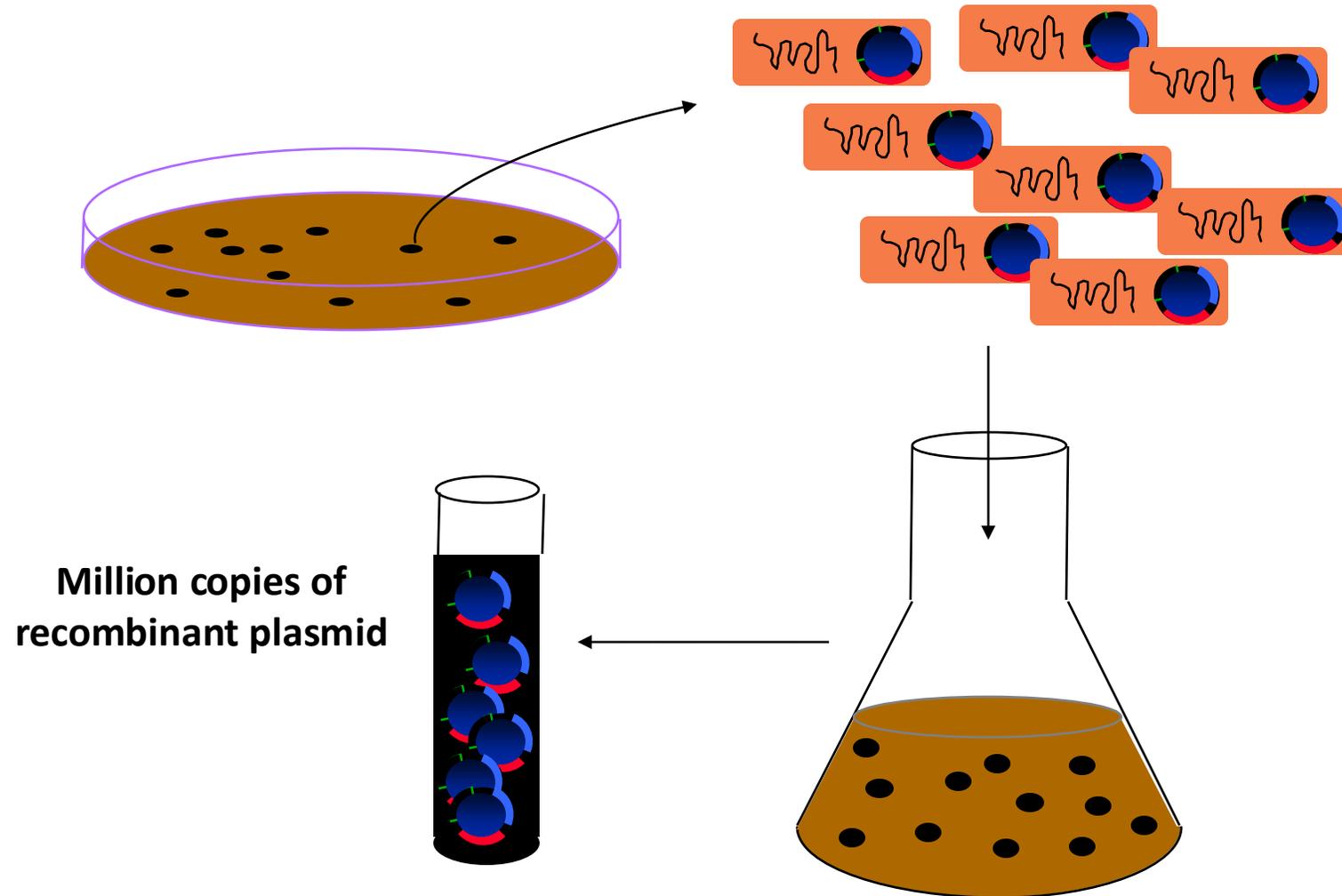


Luria Broth Agar  
+  
Ampicillin



**ONLY ANTIBIOTIC RESISTANT (AMPICILLIN) BACTERIA (PLASMID-CONTAINING)  
CAN GROW**

# DNA CLONING: MASS PRODUCTION



# DNA CLONING: PLASMIDS

**PLASMID:** These are double-stranded, circular extrachromosomal genetic elements, inside a bacterium which can separately replicate from the genome

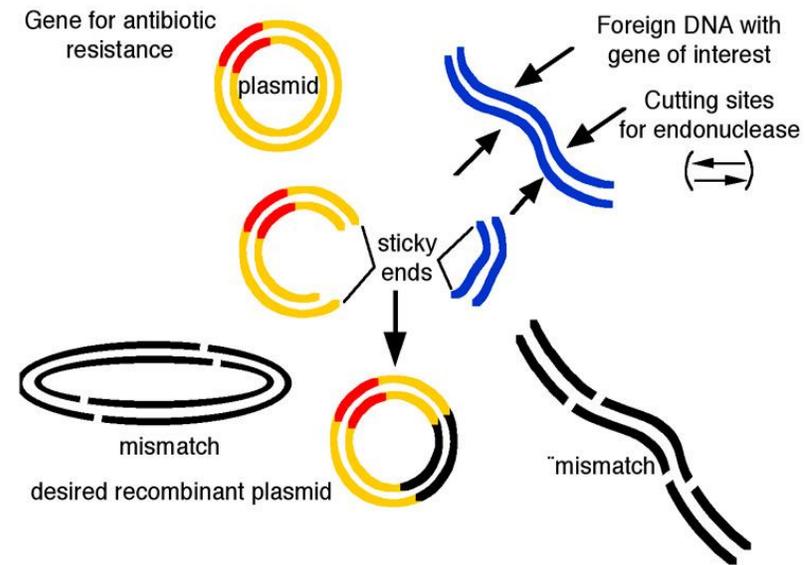
- A vector plasmid contains sequences for:
  - a **bacterial replication origin** (ori)
  - an **antibiotic resistance gene** [i.e. ampicilline resistance gene(amp)]
  - one or more special restriction digestion sites which helps the insertion of a foreign DNA fargment (MCS)
- A ***B-galactosidase*** gene losing its activity when a DNA fragment is inserted in MCS,
- **promotors** providing expression of a foreign gene in procaryotic or eucaryotic cells.

# Vector Types

- Plasmids: 15 kb capacity.
- Bacteriophages (Lambda phage): 25 kb capacity.
- Cosmid vectors: 35-45 kb capacity.
- Bacterial originated Artificial Chromosomes (BAC): 50-300 kb capacity.
- Yeast originated Artificial Chromosomes (YAC): 300-1500 kb capacity.
- Human originated Artificial Chromosomes (HAC): Greater than 2000 kb capacity.

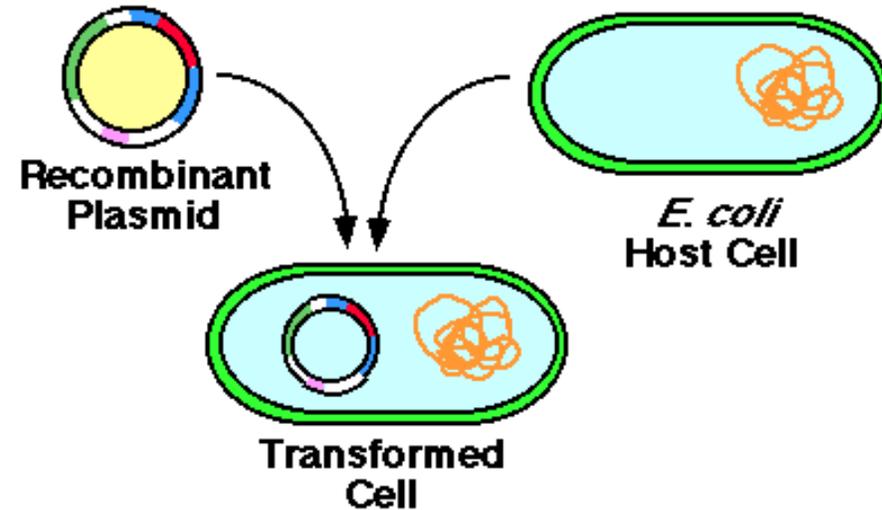
# DNA Cloning, II

- Bacterial plasmids are cut (extra-chromosomal small circular DNA structures) with the same RE.
- By this way, a DNA fragment could be inserted into plasmid DNA and a recombinant DNA molecule is formed.



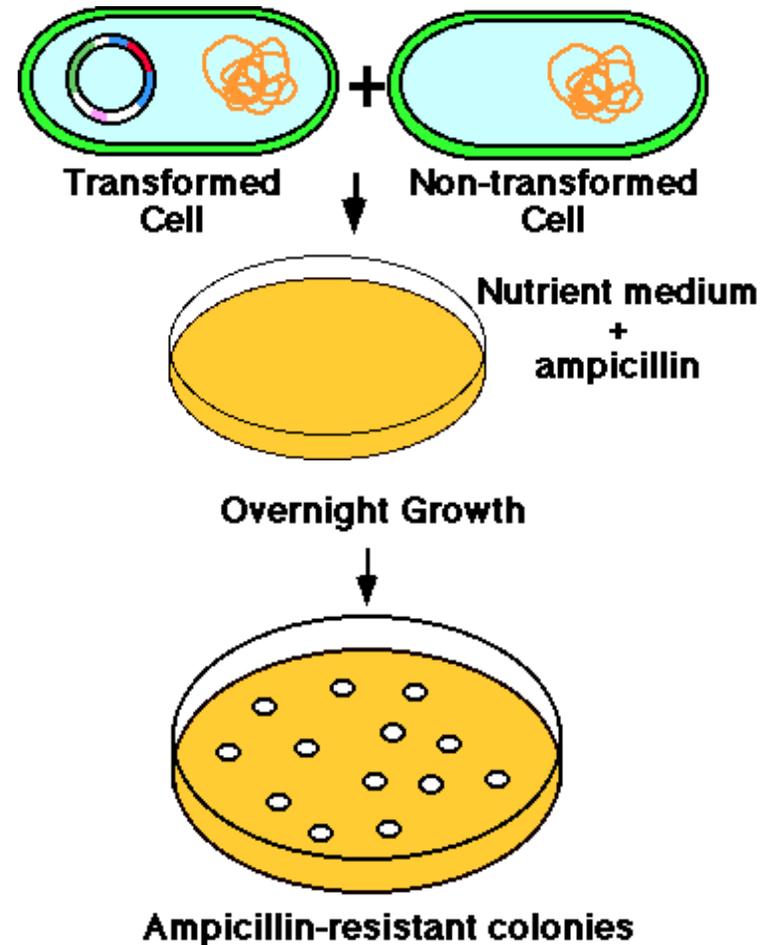
# DNA Cloning, III

- These recombinant plasmids are electroporated (put) into competent (which could take plasmids) bacteria.
- This transport or transfer process is called transformation.

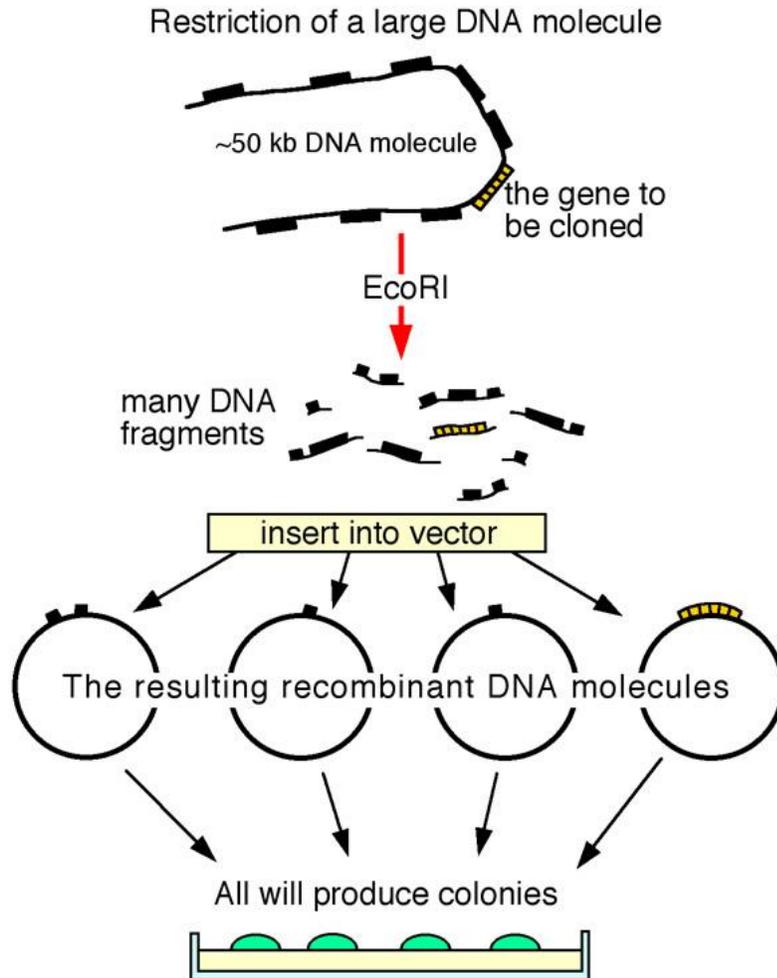


# DNA Cloning, IV

- These plasmids carry antibiotic resistance genes.
- By this way, while bacteria carrying resistance genes can grow on antibiotic containing media other bacteria are eliminated and die, thus only transformed bacteria can live and replicated on the media.



# DNA Cloning V

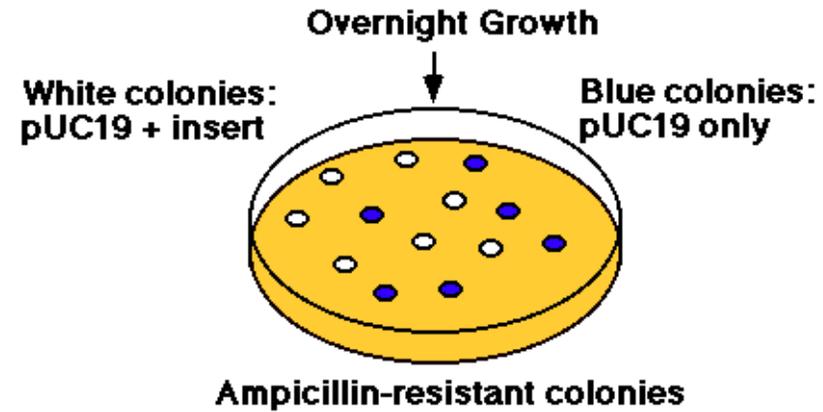


- Transformed bacteria grow on medium as colonies
- In a bacterial colony, each bacterial cell has the same plasmid thus the same DNA!
- In bacterial cells from different colonies have different plasmids thus different DNA fragments!

# Screening I

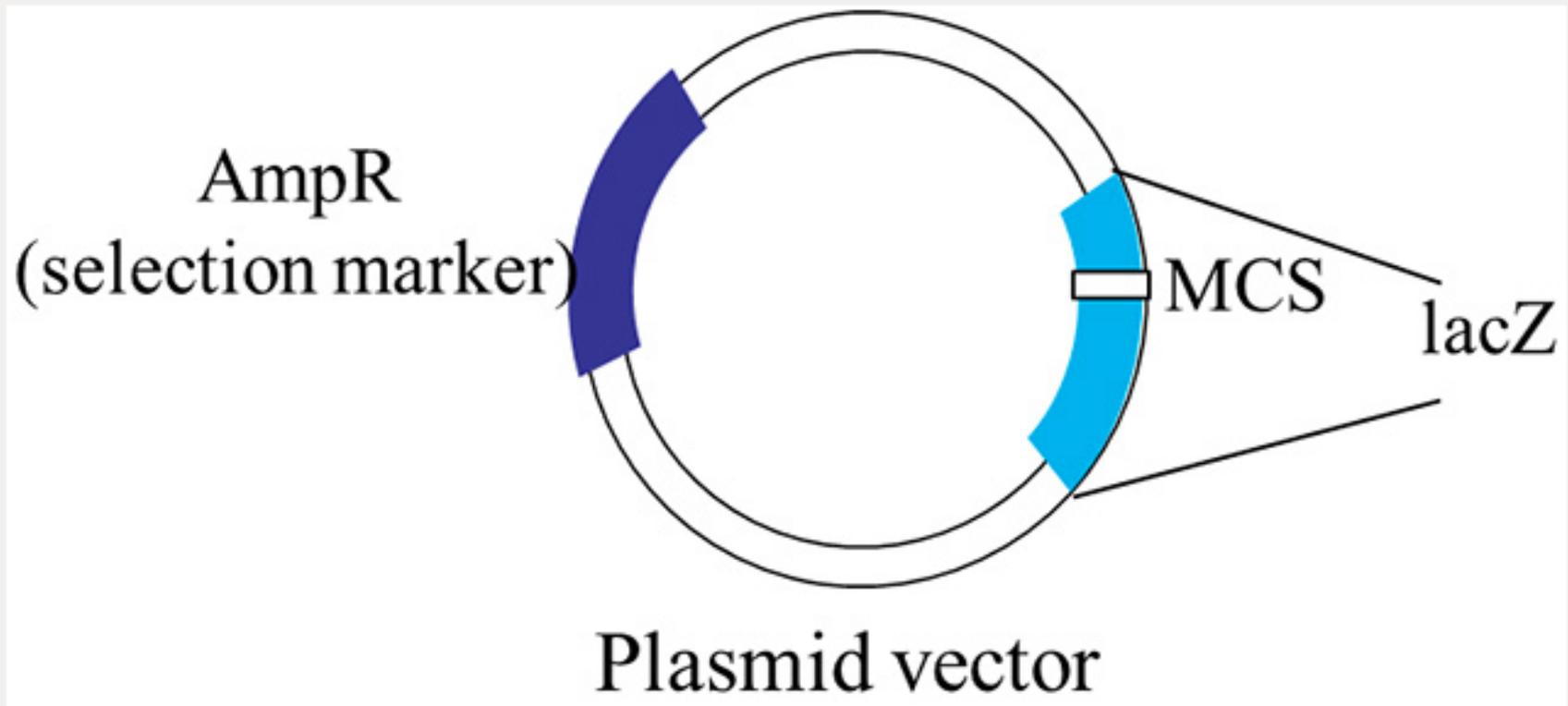
## Screening:

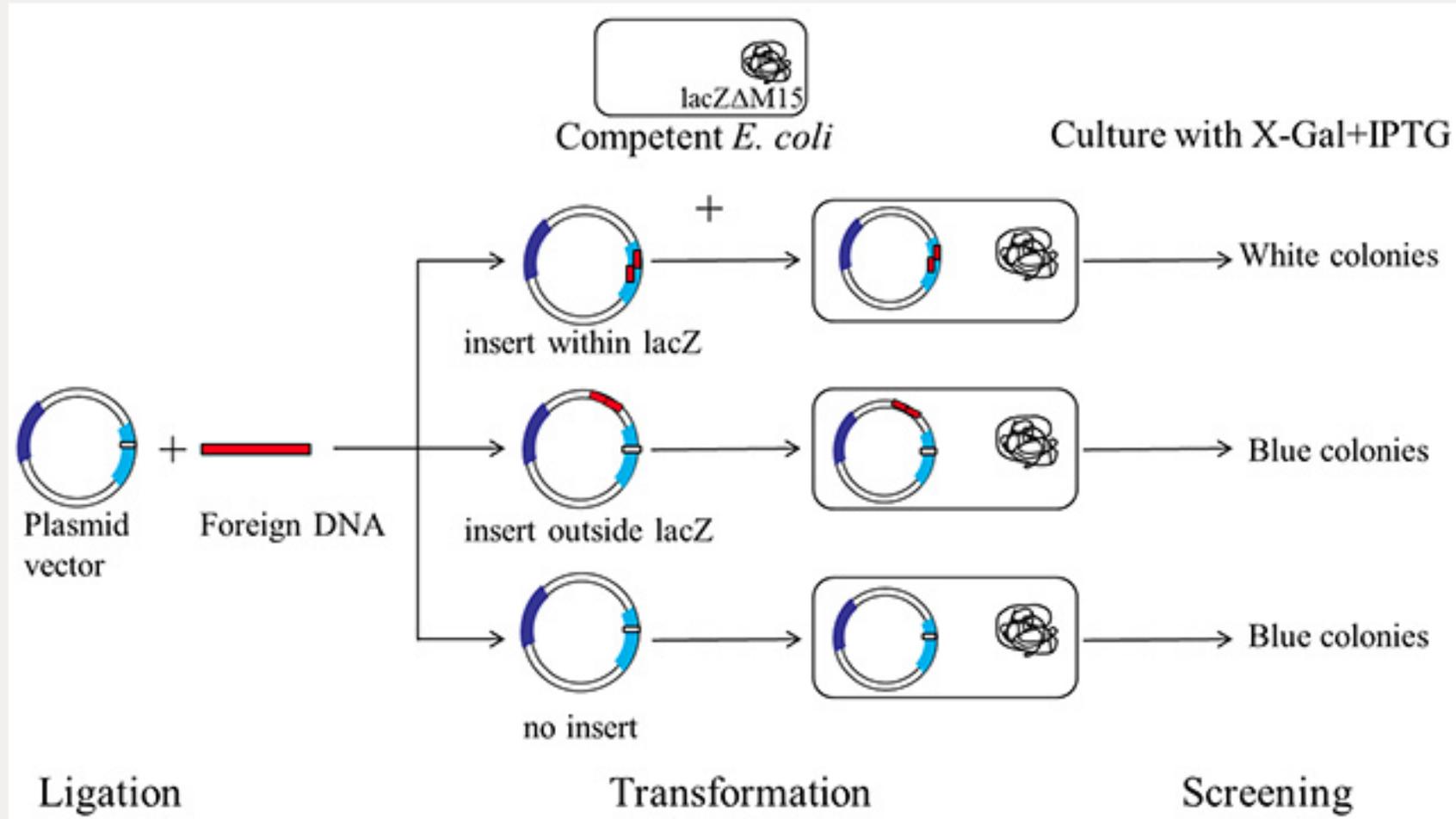
- 1. Phenotypic Screening –**  
Protein encoded by the gene present in the plasmid changes the color of the colony.
- 2. Protein expressed by a defined gene can be detected with specific antibodies**



# Blue-White Screening

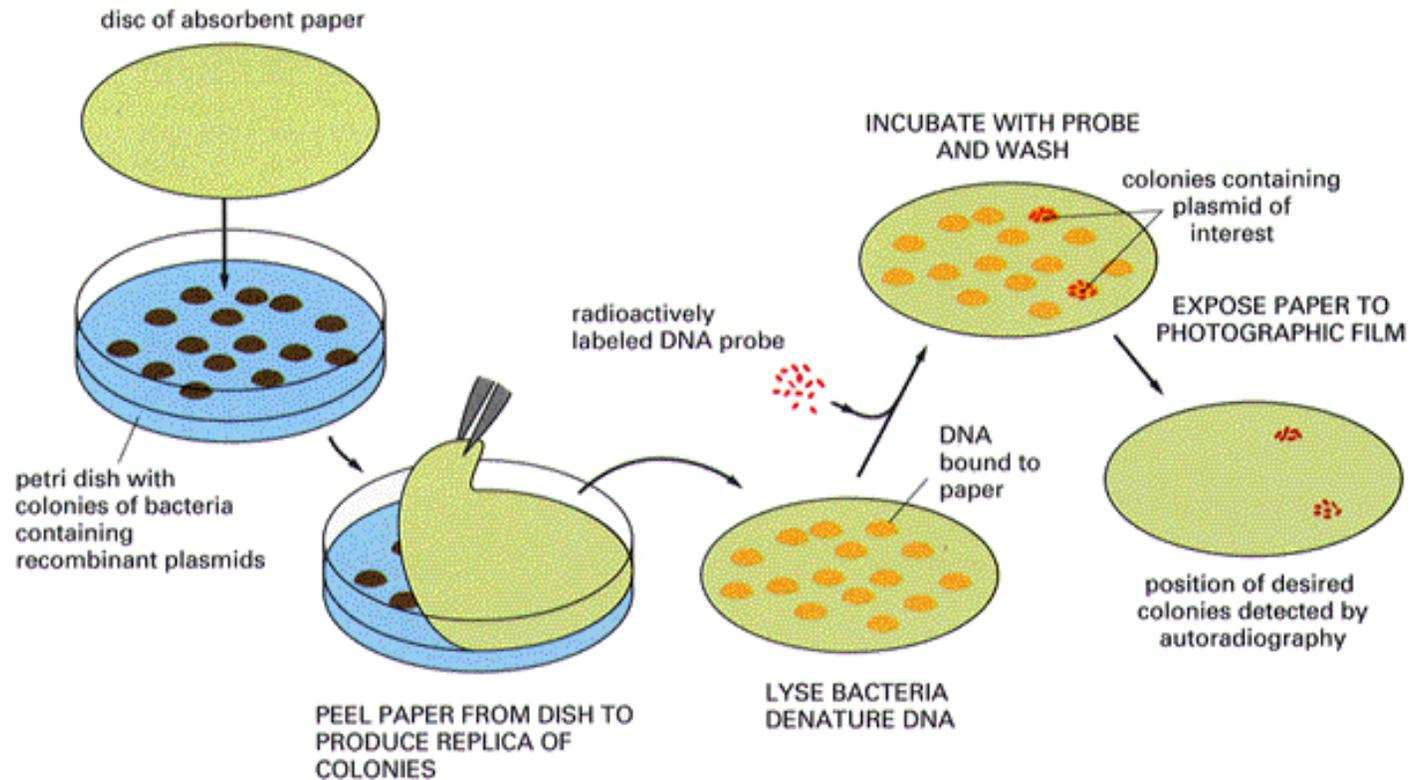
- For screening the clones containing recombinant DNA, a chromogenic substrate known as **X-gal** is added to the agar plate.
- **If  $\beta$ -galactosidase is produced**, X-gal is hydrolyzed to form 5-bromo-4-chloro-indoxyl, which spontaneously dimerizes to produce an **insoluble blue pigment** called 5,5'-dibromo-4,4'-dichloro-indigo.
- The **colonies formed by non-recombinant cells, therefore appear blue in color while the recombinant ones appear white.**
- The desired recombinant colonies can be easily picked and cultured.





# Screening II

3. DNA sequence of a cloned gene can be detected with a DNA hybridisation probe.



# Screening III

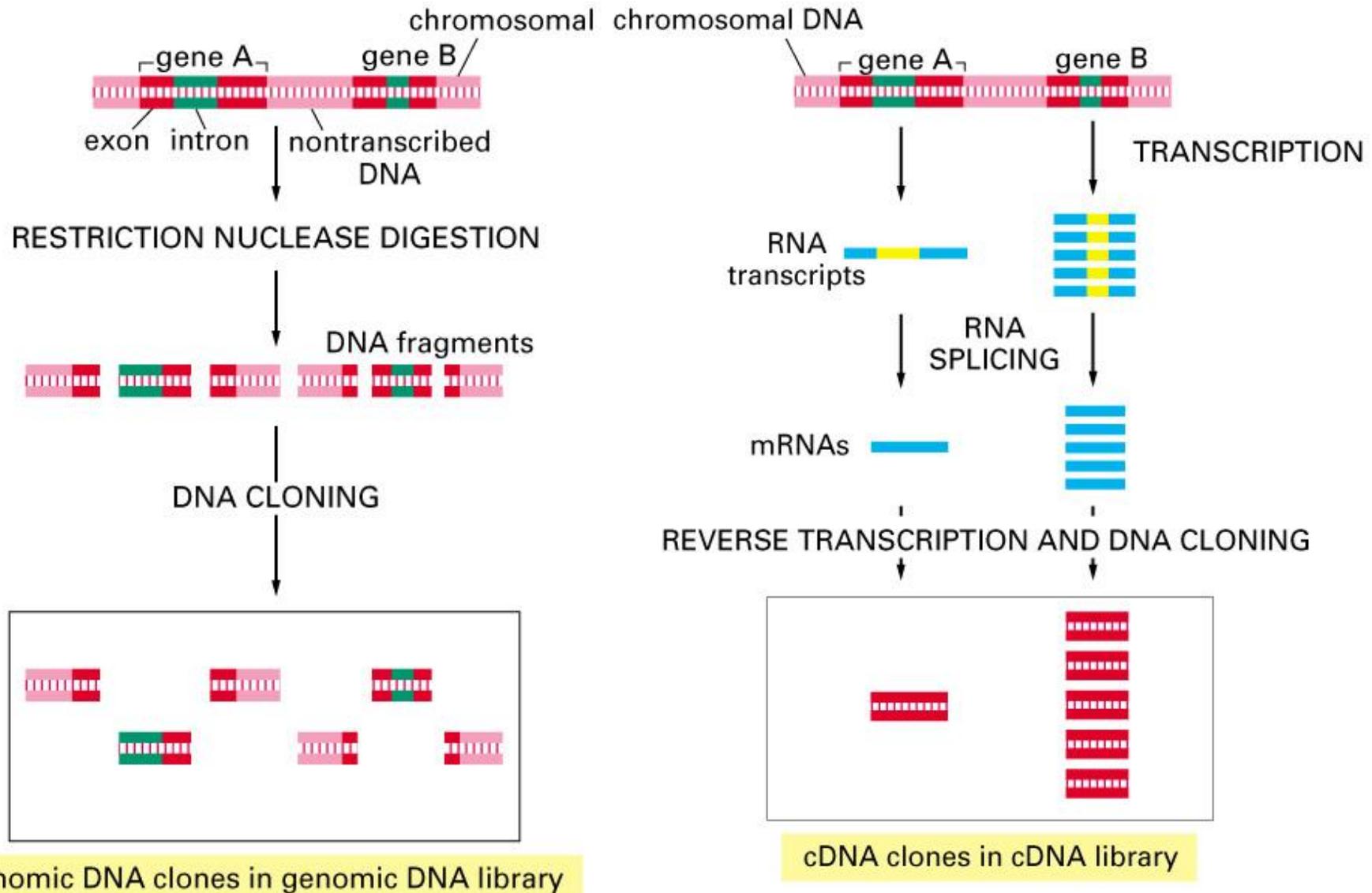
- Following the screening and isolation of colonies they can mass produced by culturing in broths!
- These can be stored at -80°C for years!



# DNA Libraries

- DNA Library: In molecular biology, collection DNA fragments produced and stored in microbial populations are called DNA libraries!!!
- They are collection of living bacterial colonies transformed with different DNA fragments obtained from different organisms those form source of DNAs
- These gene libraries are screened and researchers work with colonies carrying the genes of interest!
- There are also **cDNA libraries** and **gene libraries** established from genomic DNA!

# Clone Libraries



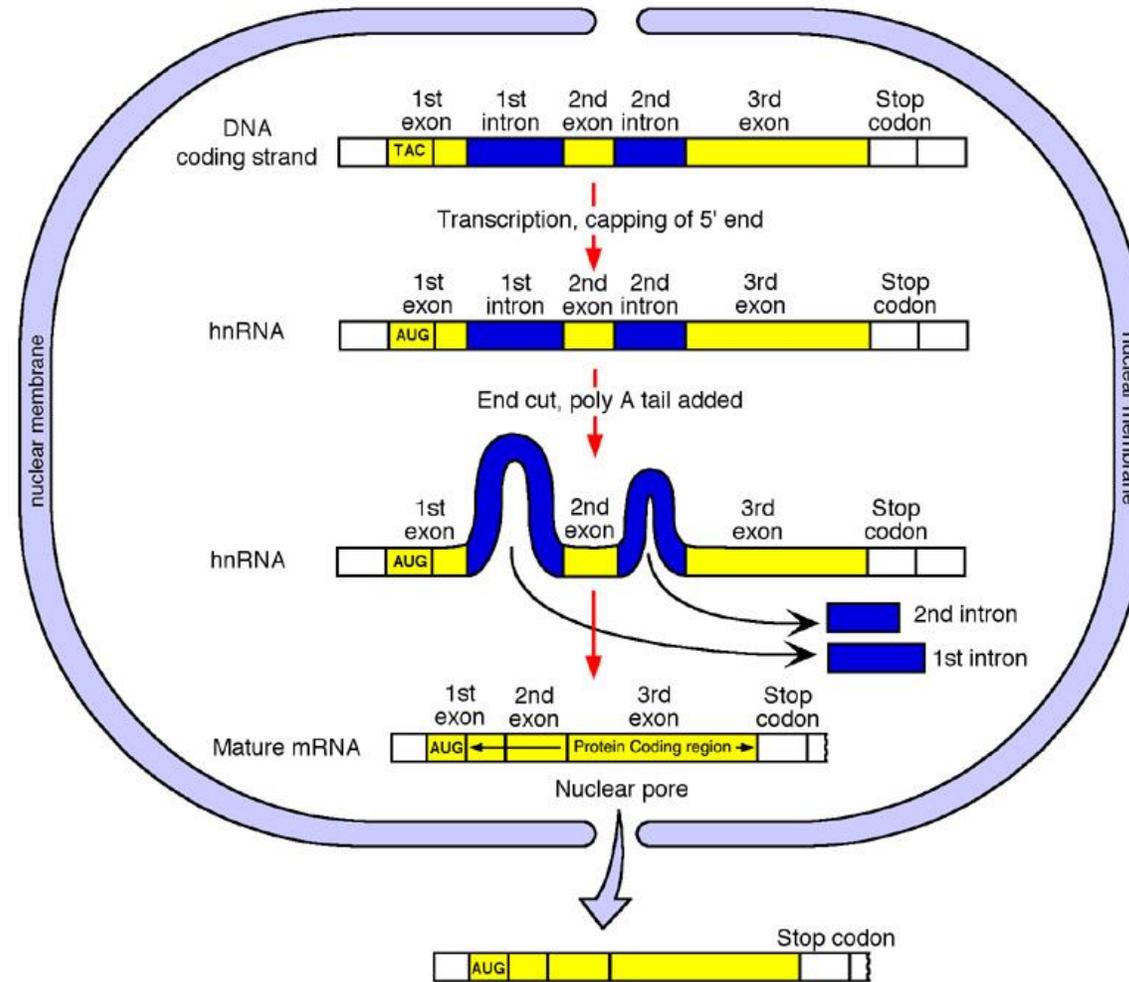
# cDNA ve Gene Library Applications

## Why do we need them?

- For identification of new genes
- *In vitro* investigation of gene functions (cDNA molecule cloning)
- mRNA expression analysis from diverse cell and tissues
- Whole genome identification of specific organisms i.e. (human genome project or other genome projects)
- Establishment of genomic sequence resources for the production of transgenic animals
- *In vitro* investigation of regulatory sequence functions
- Investigation of genetic mutations in cancer tissues

# cDNA I

- Eucaryotic DNA is different from a bacterial (procaryotic) DNA, since it has **intron** (intervening sequences) ve **exon** (expressed or translated sequences) sequences.
- In order for an eucaryotic gene to be expressed, introns should be excised from mRNA following transcription!



Transcription in Eucaryotes!!!

# CENTRAL DOGMA and GENE CLONING

