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# **Restriction Endonucleases (Molecular Scissors)**

**By**

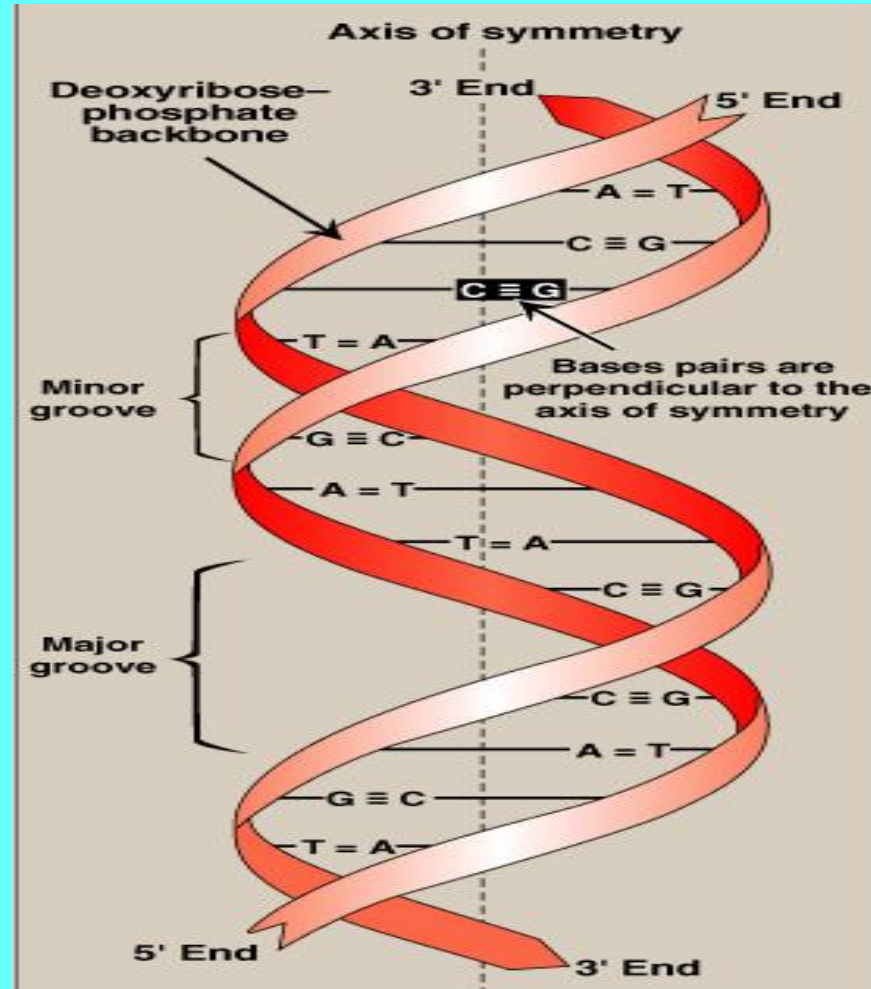
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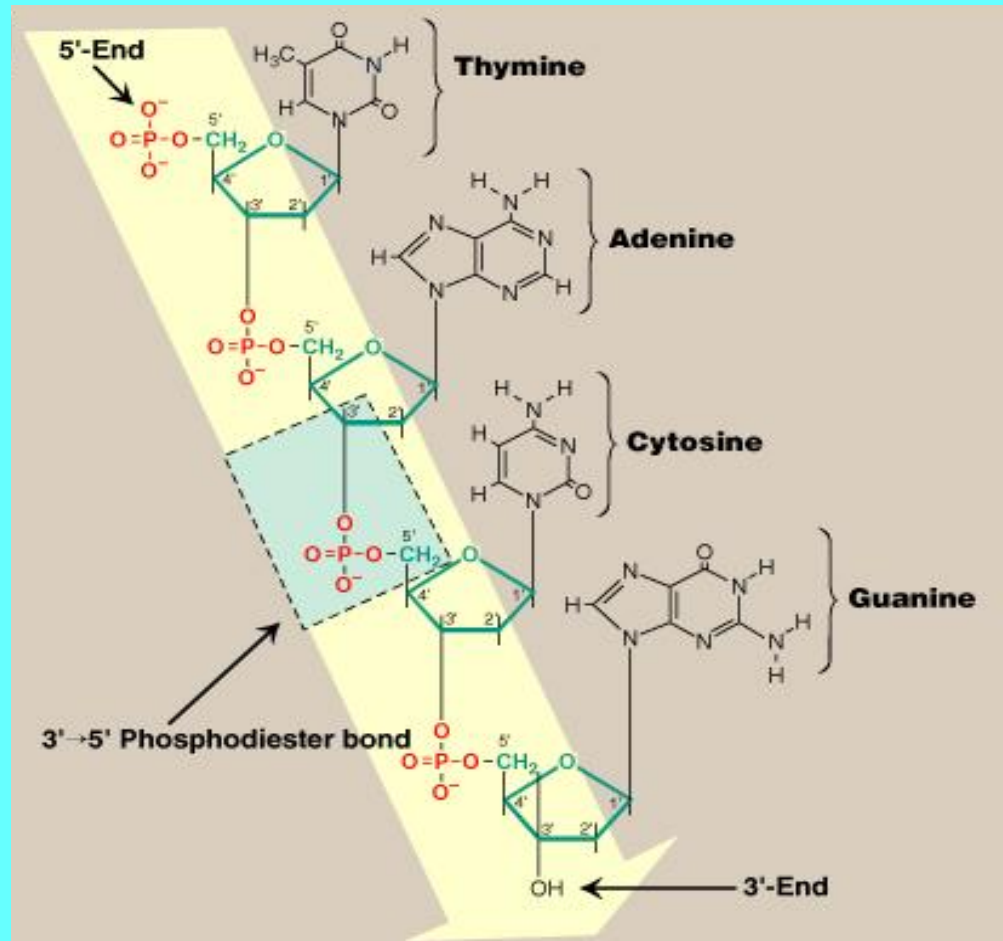
# Objectives

- **Endonucleases Vs Exonucleases**
- **Recognition sequences**
- **Sticky Vs Blunt ends**
- **Reaction conditions**
- **Electrophoretic detection**
- **Applications**

# Background information: dsDNA



# Background information: 3'-5' Phosphodiester Linkage



# Endonucleases Vs Exonucleases

Exonucleases cleave from the end of the chain, releasing single nucleotides.



Endonucleases cleave within the chain, producing nicks

# Restriction Endonucleases

**Origin:**

**Bacterial enzymes**

**Binomial nomenclature:**

*Eco* RI; *Hae* III

**Recognition sequences:**

**Palindrome**

**4- or 6- base pairs**

# Restriction Endonucleases

They cleave dsDNA: Sequence-dependent

Palindromic sequence:

**Refer**

**Level**

**Madam I'm Adam**



**Refer**

**Level**

**madam I'm Adam**

Restriction sites

Restriction fragments



# DNA Recognition Sequence: A Palindrome

## A Palindrome

When read in the 5' → 3' direction, the sequence on the “top” strand is identical to that of the “bottom” strand .

5' -GAATTC- 3'

3' -CTTAAG- 5'

# Restriction Endonucleases: Sticky Vs Blunt ends

## Sticky (cohesive) ends

5' Single-stranded overhang, e.g., *Bam* HI



3' Single-stranded overhang, e.g., *Kpn*I



# Restriction Endonucleases: Sticky Vs Blunt ends

## Blunt (Flush) ends

Cleavage at the axis of symmetry, e.g., *Hae* III



# **Restriction Endonucleases: Reaction Conditions**

- **Ionic strength: 100-150 mM**
- **pH: < 8.0**
- **Divalent cation: Mg<sup>2+</sup>**
- **Glycerol contents: < 5% (V/V)**
- **Units of enzyme: to amount of DNA**
- **Temperature: 37 °C**

# **Restriction Endonucleases: Reaction Conditions - 2**

- **DNA Digestion with multiple enzymes**
- **Inactivation of the enzymes**
- **Star activity**

# **Restriction Endonucleases: Electrophoretic Detection**

**DNA sequence (restriction site)**

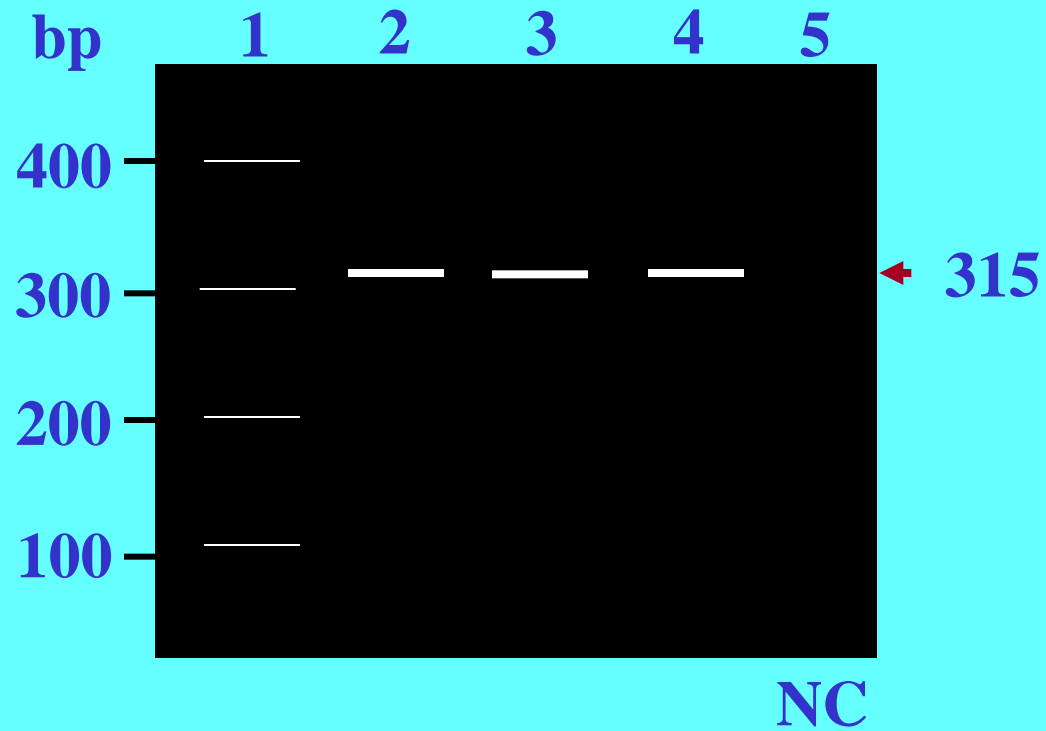
**Restriction enzyme**



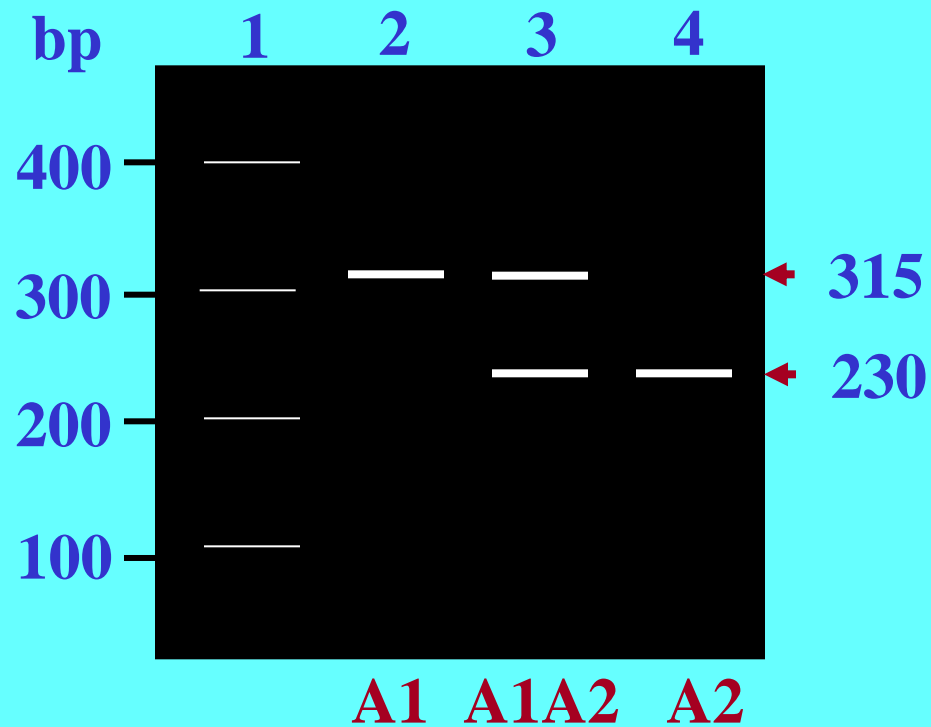
**DNA Restriction fragments**

**Detection by DNA gel electrophoresis**

# PCR of SA Gene



# SA Genotypes





# **Restriction Endonucleases: Applications**

- **Production of Recombinant DNA & Cloning**
- **Production of DNA & cDNA Libraries**
- **Analysis of DNA: e.g., Southern blotting**
- **Detection of mutations:  
e.g., Diagnosis of sickle cell anemia by RFLPs**

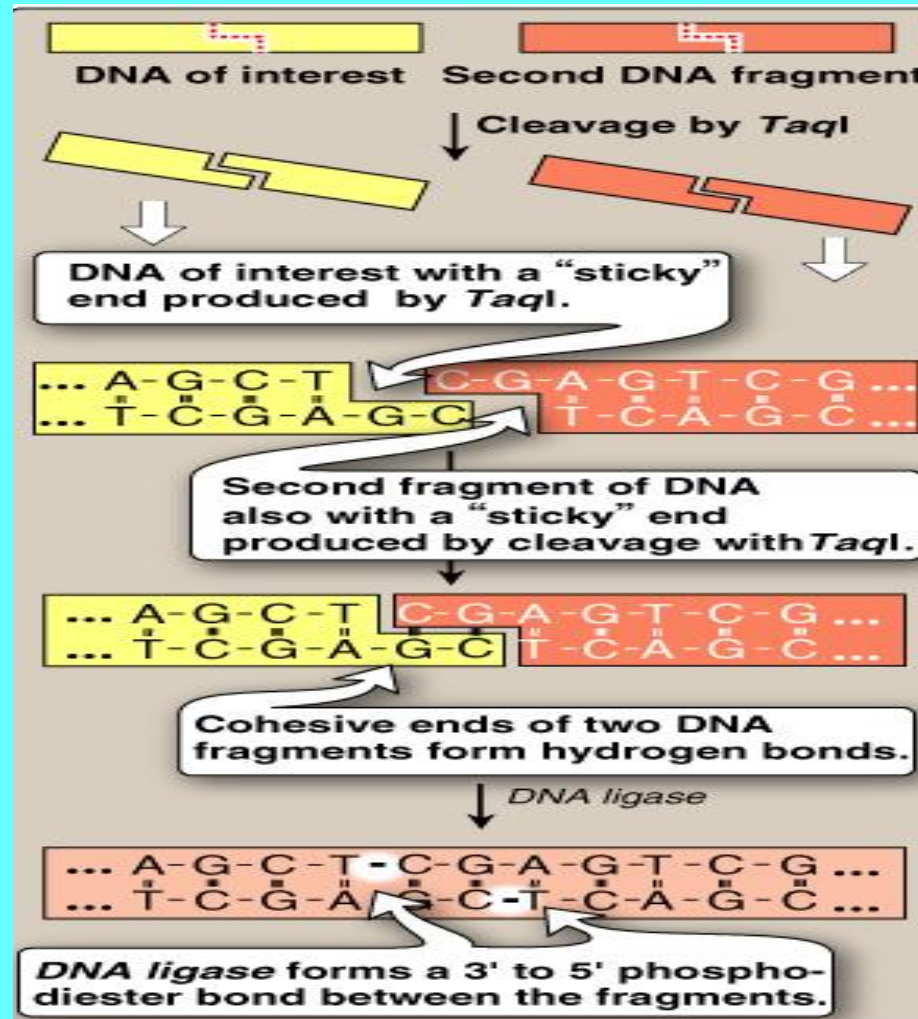
# **DNA Cloning**

**Recombinant DNA Construct:  
Target DNA plus Vector**

**Living (replicating ) cells**

**Amplified target DNA**

# Production of Recombinant DNA



# Recombinant DNA - 2

## ➤ Target DNA sequence

- DNA
- cDNA
- Synthetic DNA

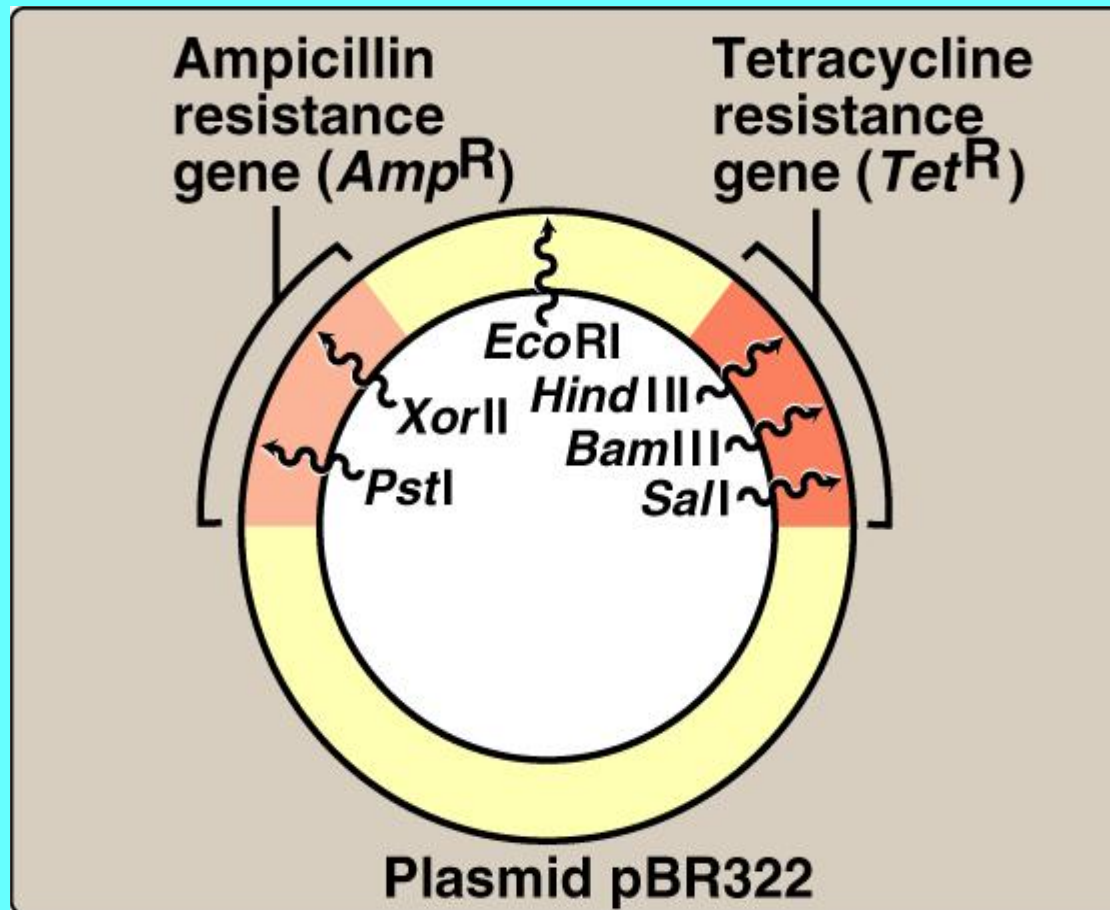
## ➤ Vectors

- Plasmids
- Others: Bacteriophage, Cosmids, BACs, YACs, Mammalian cells

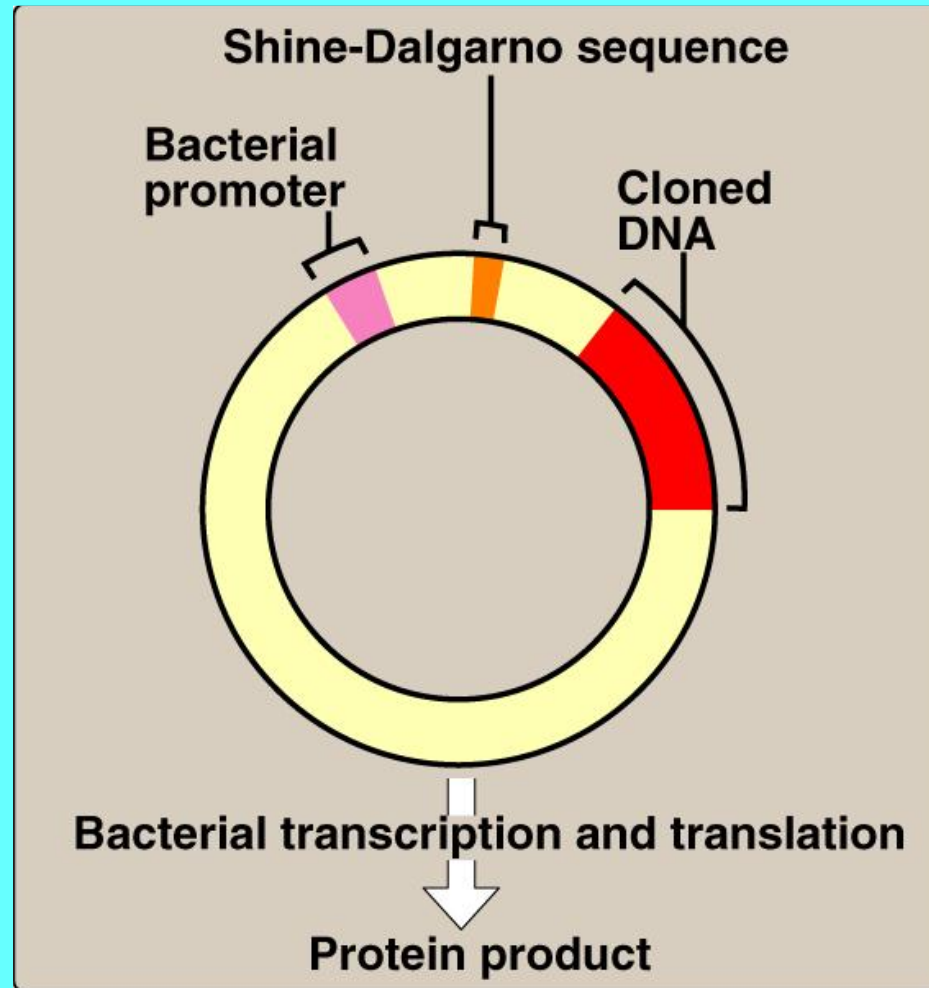
# Plasmid Vector

- **Common features:**
  - **Origin of replication (ori)**
  - **Selectable marker**
  - **Cloning site(s)**
- **Additional elements (Expression vectors):**
  - **Transcriptional promotor, inducible**
  - **Translational control sequence**
    - **ATG start codon**
    - **Stop codons**
  - **Coding sequence for fusion protein**

# Plasmid Vectors



# Plasmid Expression Vectors



# Recombinant DNA Assembly

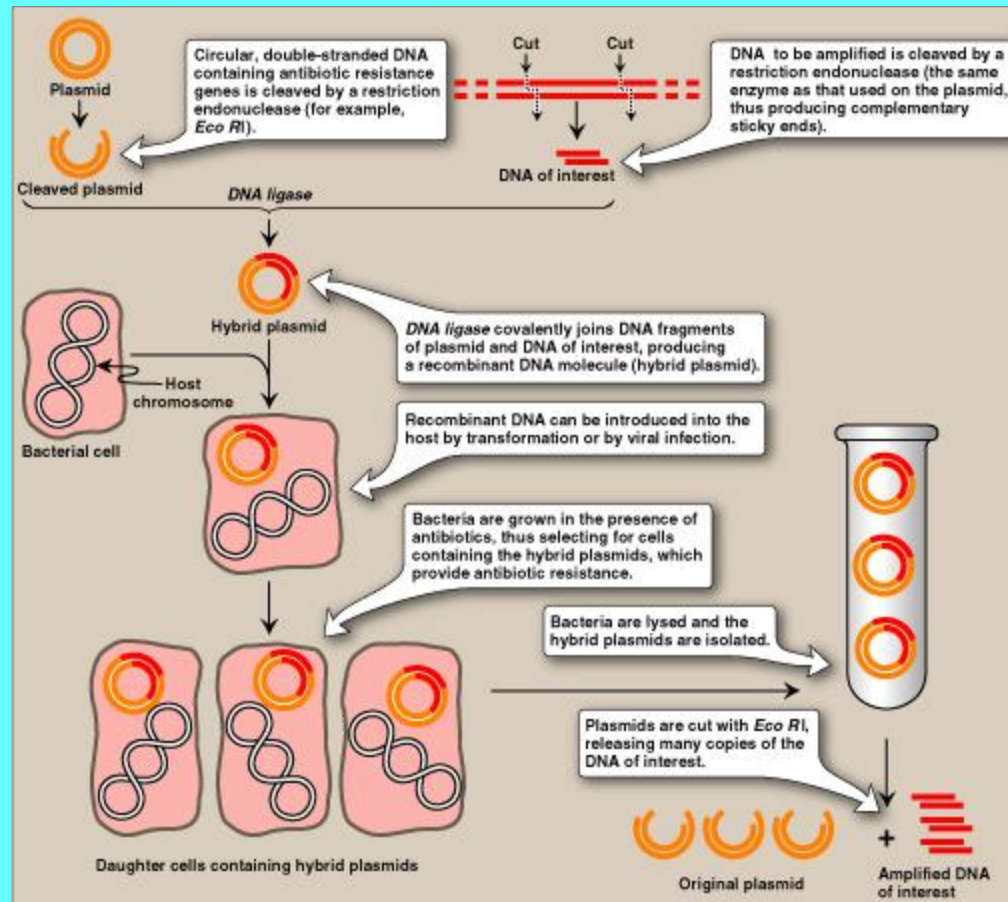
- **DNA modifying enzymes:**
  - **Restriction endonucleases**
  - **DNA polymerases**
  - **DNA kinases**
  - **Alkaline phosphatases**
  - **DNA ligases**
- **Synthetic linkers and adaptors**



# Bacterial Transformation

- Introduction of foreign DNA into **competent** bacterial host
- Chemical or electroporation
- Screening for target bacterial clone

# Summary for DNA Cloning



# Protocol for DNA Cloning

- Assemble recombinant DNA construct
- Prepare competent *E. coli* strain
- Transform *E. coli* strain
- Screen transformants for target clone
- Confirmation: Miniprep & restriction map

Thank you

