بسم الله الرحمن الرحيم

Prokaryotic Gene Expression and Recombinant Protein Production

By

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Revision: Restriction Enzymes

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

Restriction Endonucleases

Origin:

Bacterial enzymes

Binomial nomenclature:

***Eco* RI; *Hae* III**

Restriction Endonucleases

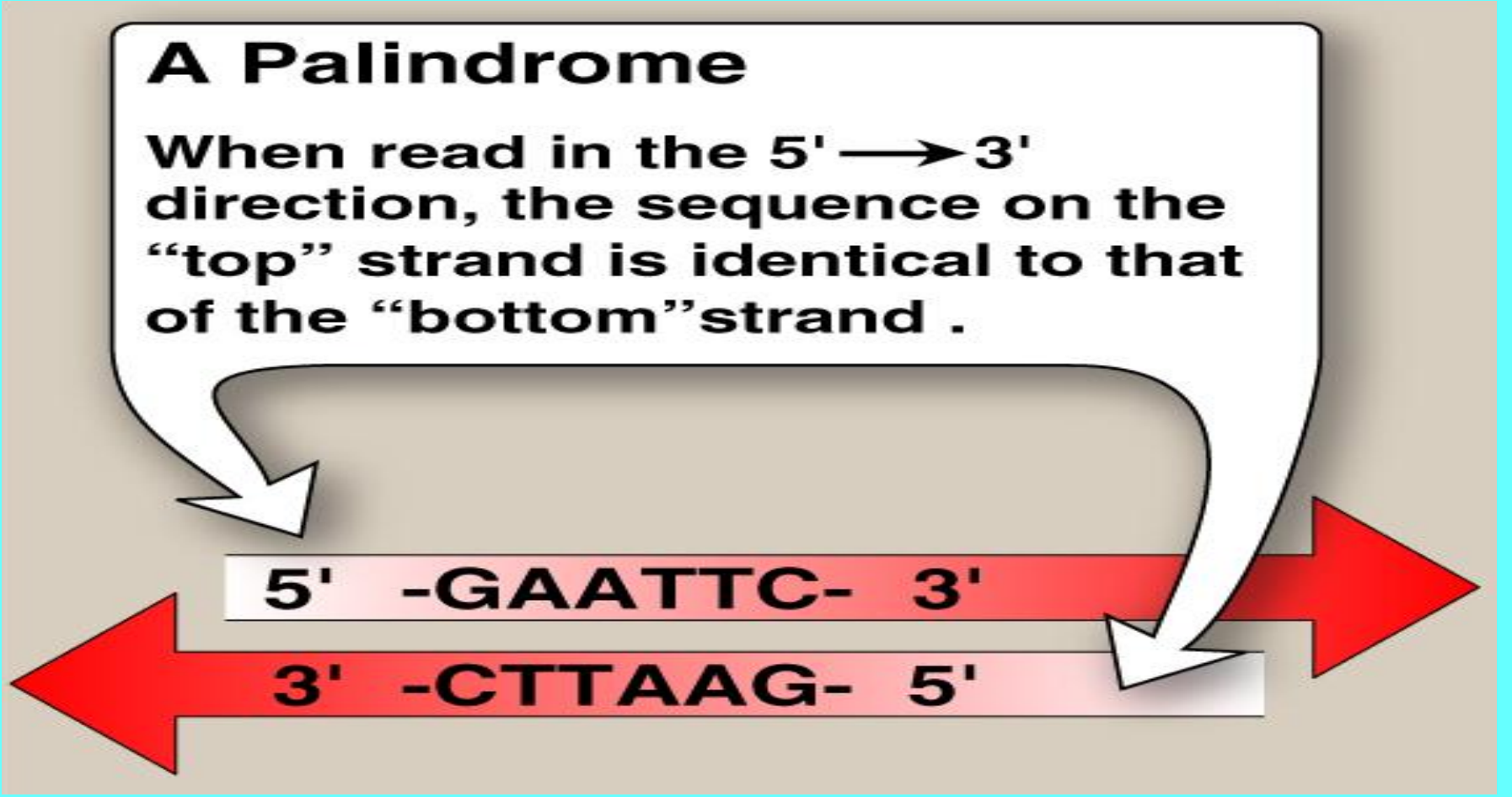
Restriction sites:

- dsDNA, palindromic sequence
- 4- or 6- base pairs
- Sticky or blunt ends

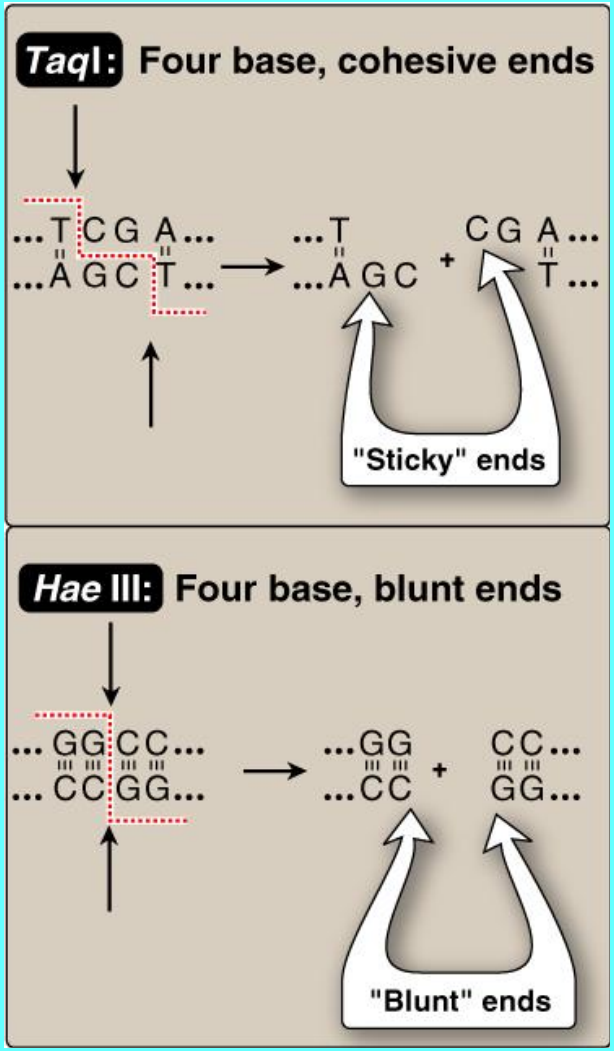
Restriction fragments:

- Detected by agarose gel electrophoresis
- Size as compared to DNA size markers

DNA Recognition Sequence: A Palindrome



Restriction Endonucleases: Sticky Vs Blunt ends



Restriction Endonucleases: Reaction Conditions

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

Restriction Endonucleases: Reaction Conditions - 2

- DNA Digestion with multiple enzymes

- Inactivation of the enzymes:
  - Heat inactivation
  - EDTA inactivation

Restriction Endonucleases: Electrophoretic Detection

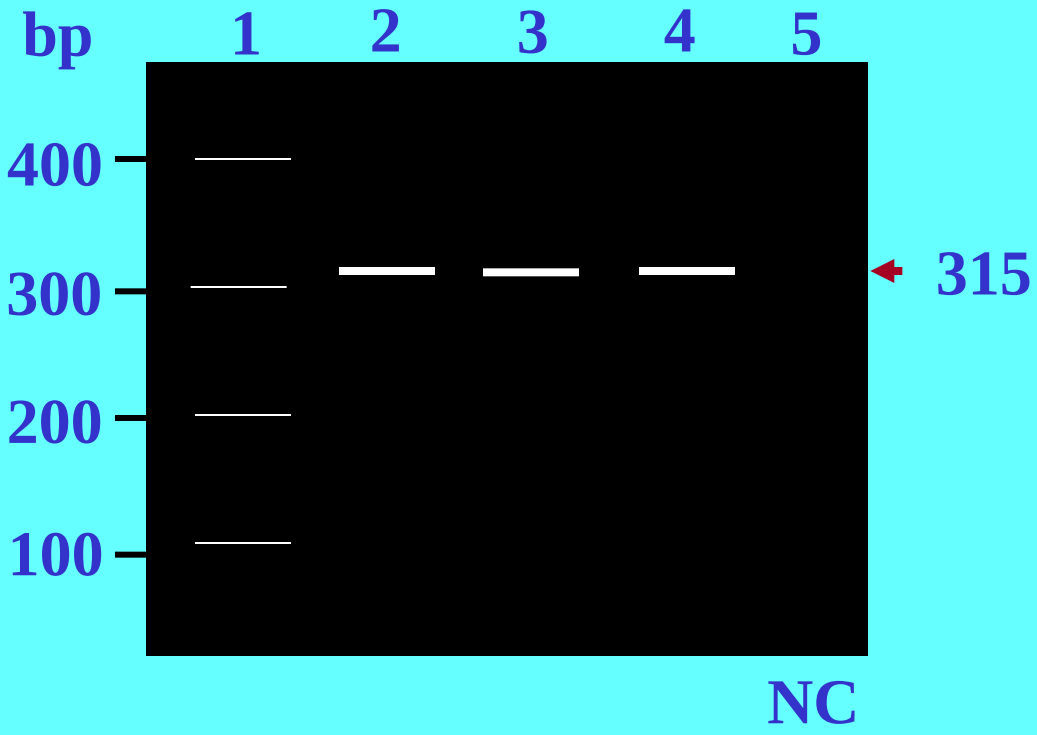
DNA sequence (restriction site)

Restriction enzyme

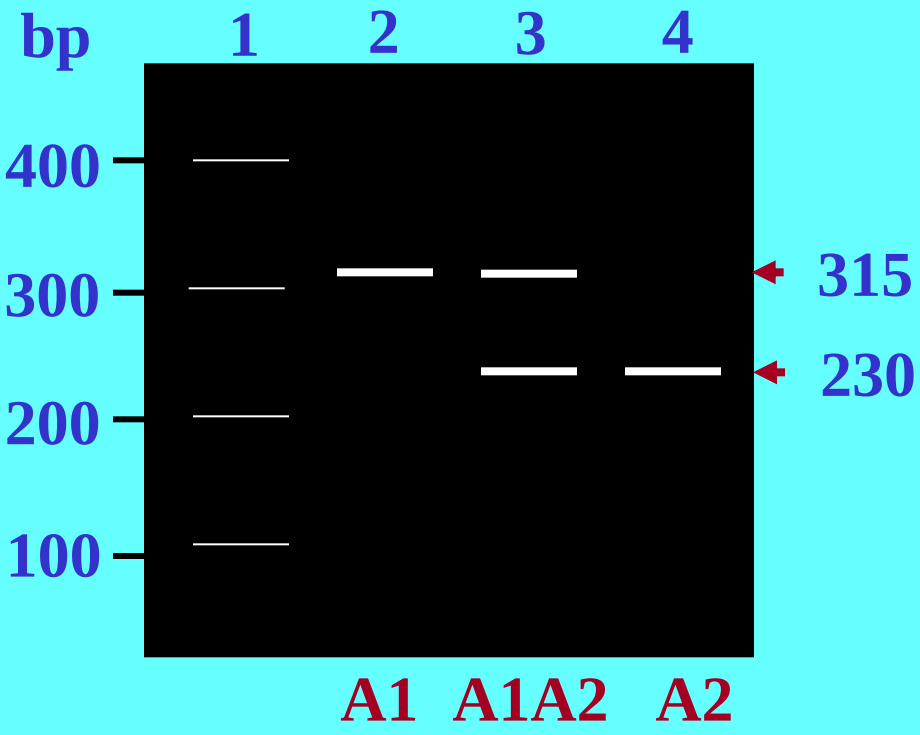
DNA Restriction fragments

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

Objectives: DNA Cloning and Recombinant Proteins

- Production of Recombinant DNA
- Bacterial transformation
- Screening for target clone
- Prokaryotic gene expression protocol
- Uses of recombinant proteins

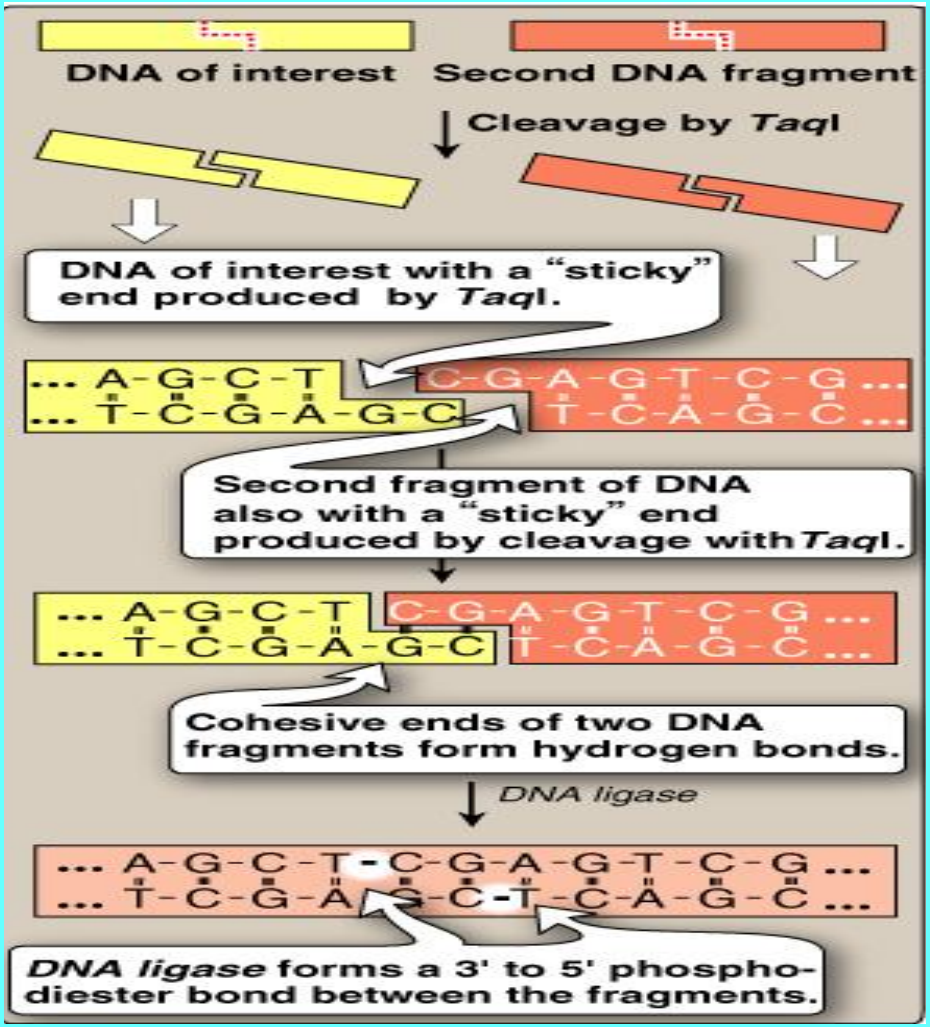
DNA Cloning

Recombinant DNA Construct:
Target DNA plus Vector

Living (replicating) cells

Amplified target DNA

Production of Recombinant DNA



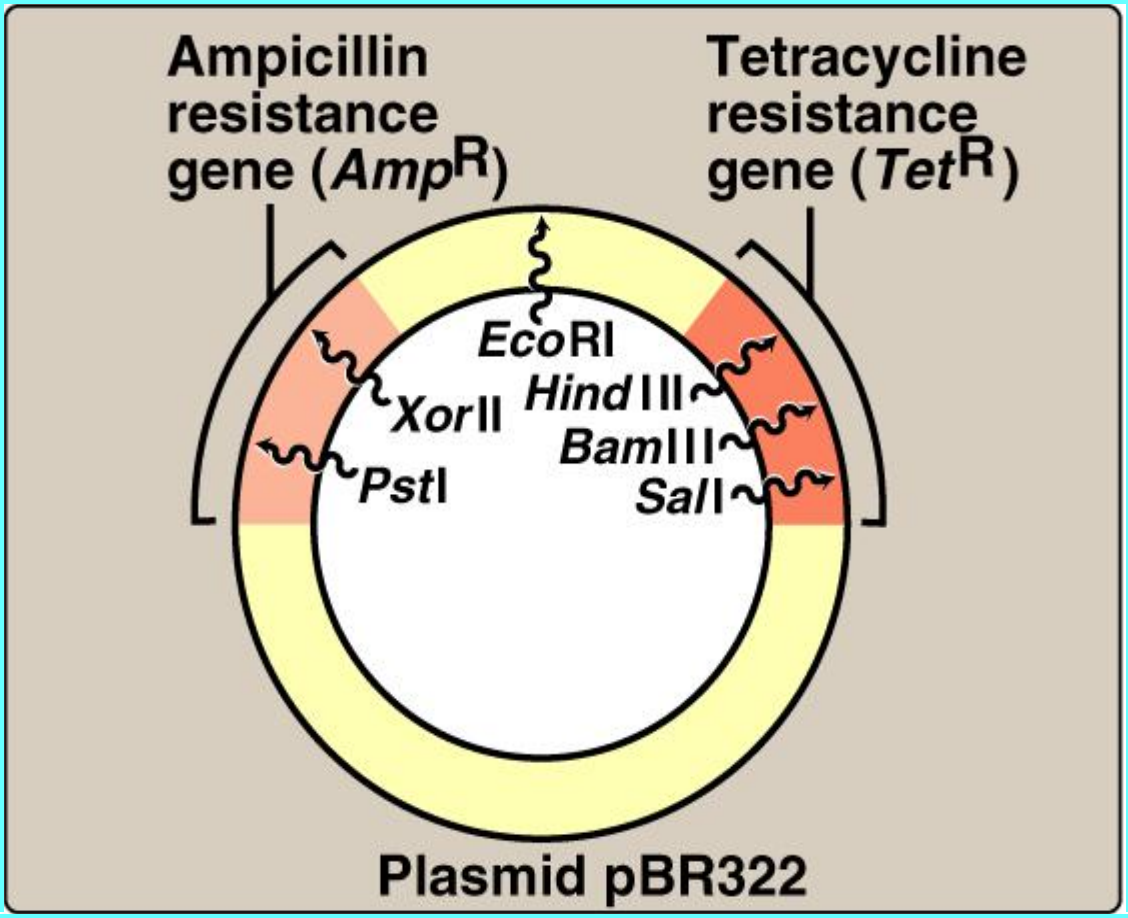
Recombinant DNA - 2

- **Target DNA sequence**
  - **DNA**
  - **cDNA**
  - **Synthetic DNA**
- **Vectors**
  - **Plasmids**
  - **Others: Bacteriophage, Cosmids, BACs, YACs, Retroviruses**

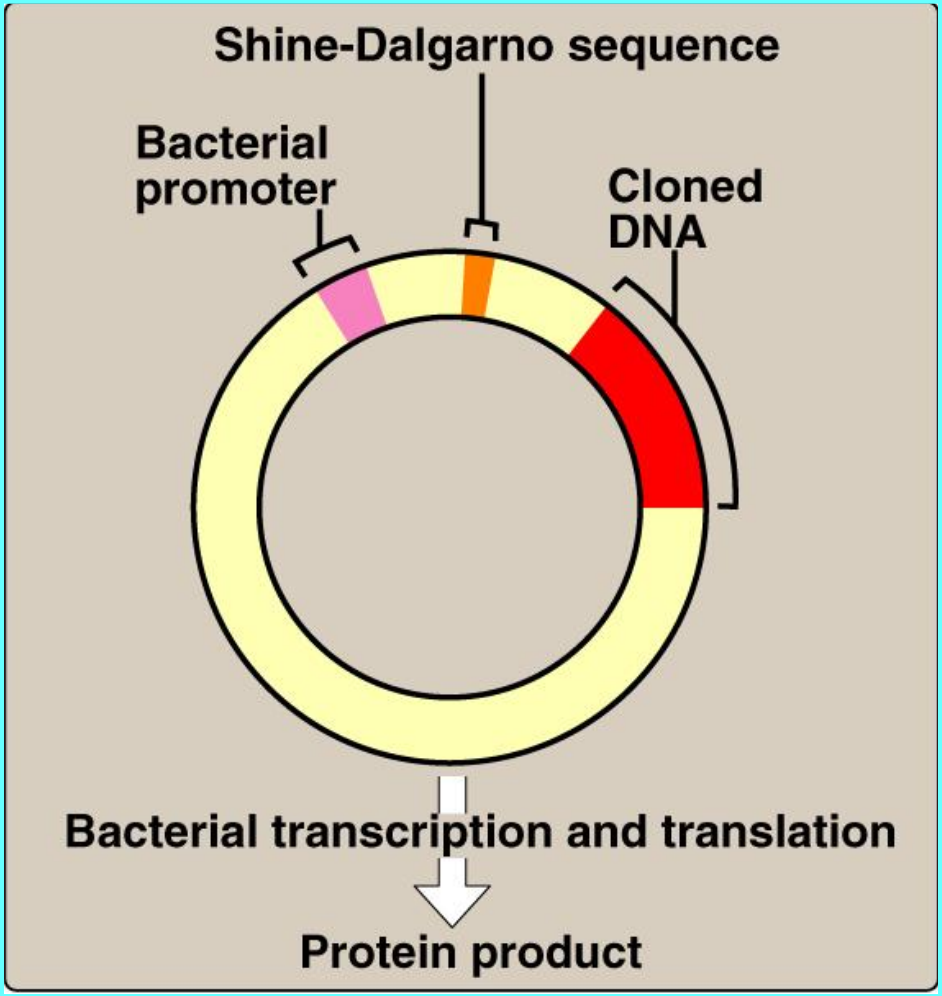
Plasmid Vector

- **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



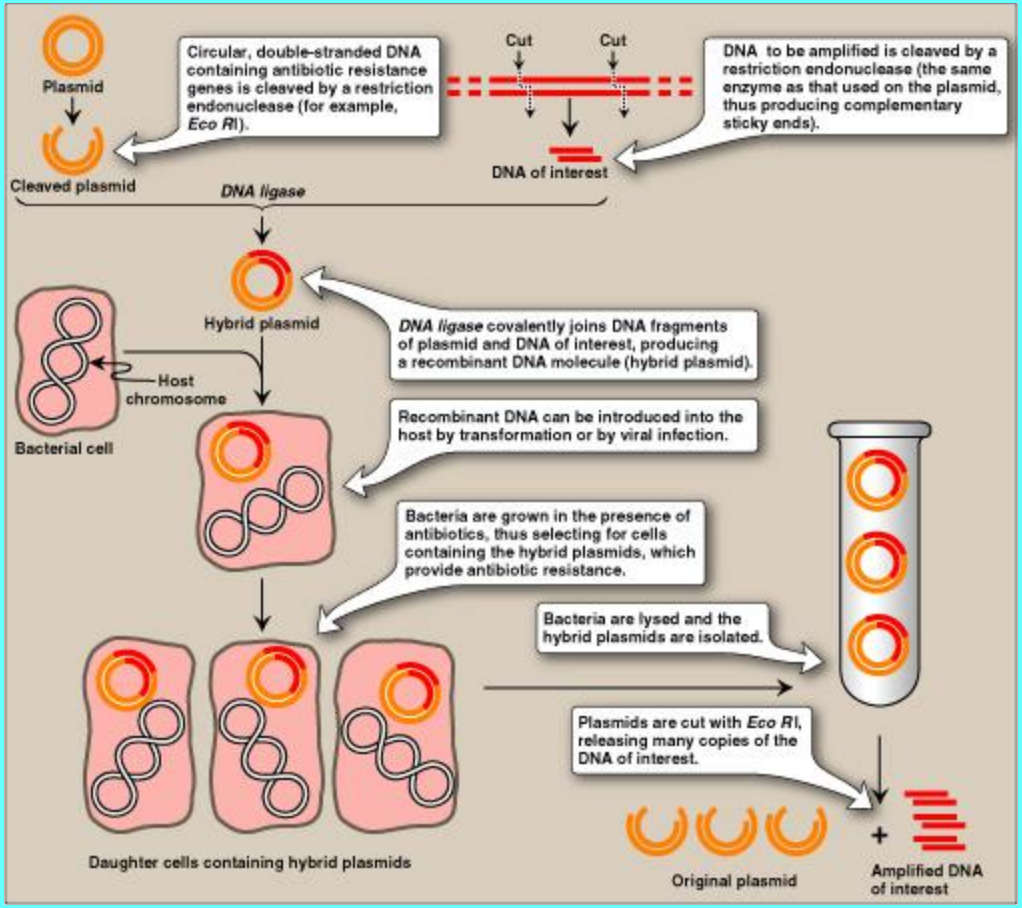
Recombinant DNA Assembly

- **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**

Prokaryotic Gene Expression

- **Definition:**

  Directed synthesis of gene-encoded protein in living bacterial cells

- **Goals:**
  - **Research:**

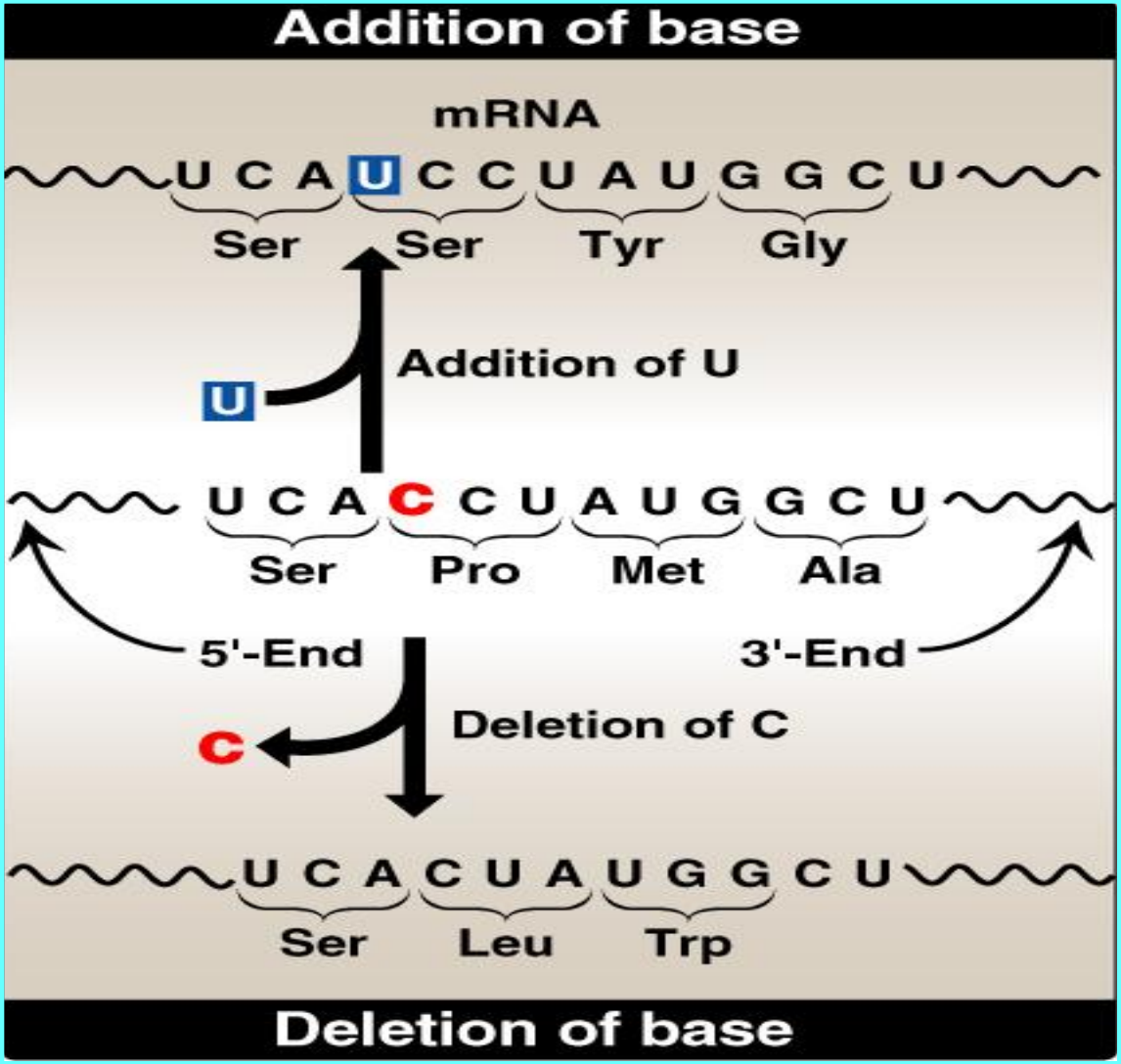
    Structure-function relationship

    Production of antibodies
  - **Medical, pharmaceutical & industrial**

Expression Construct: Assembly

- DNA modifying enzymes
- Synthetic linkers and adaptors
- In-frame ligation of insert & vector DNAs

The Open Reading Frame



E coli Gene Expression - 1

- **Advantages:**
  - **Relatively simple & achievable in short time**
  - **Efficient: expressed protein ~30% of total cellular proteins**
  - **Cheap**
- **Disadvantages**
  - **Euokaryotic proteins: not properly modified**
  - **Inclusion bodies**
  - **Toxic genes**

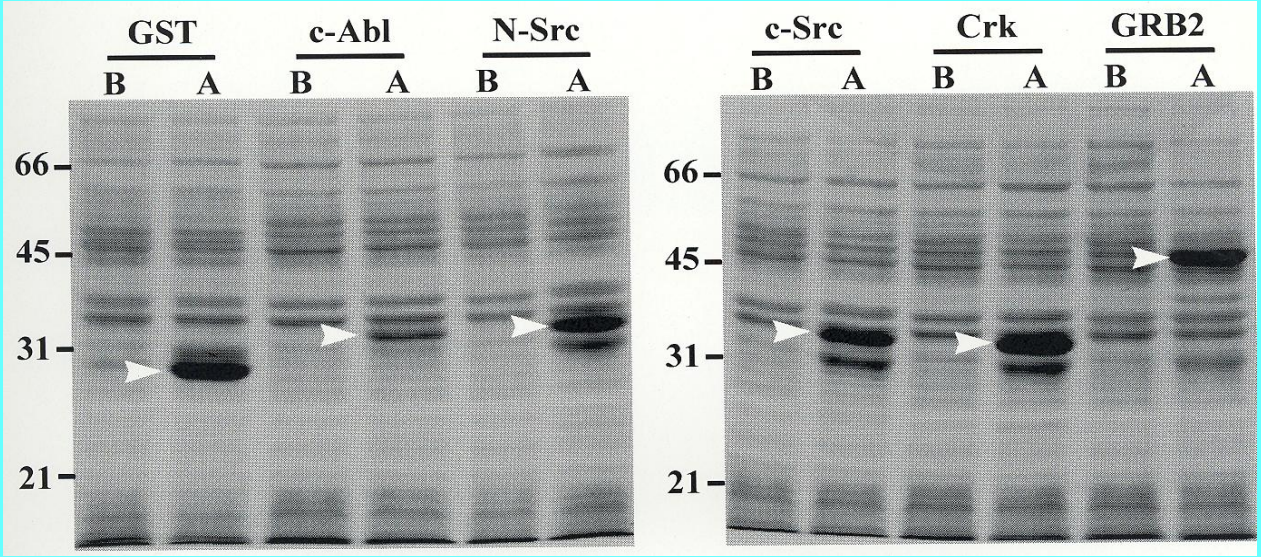
E. coli Gene Expression - 2

- Direct expression system
- Fusion protein expression system
- Secretory expression system

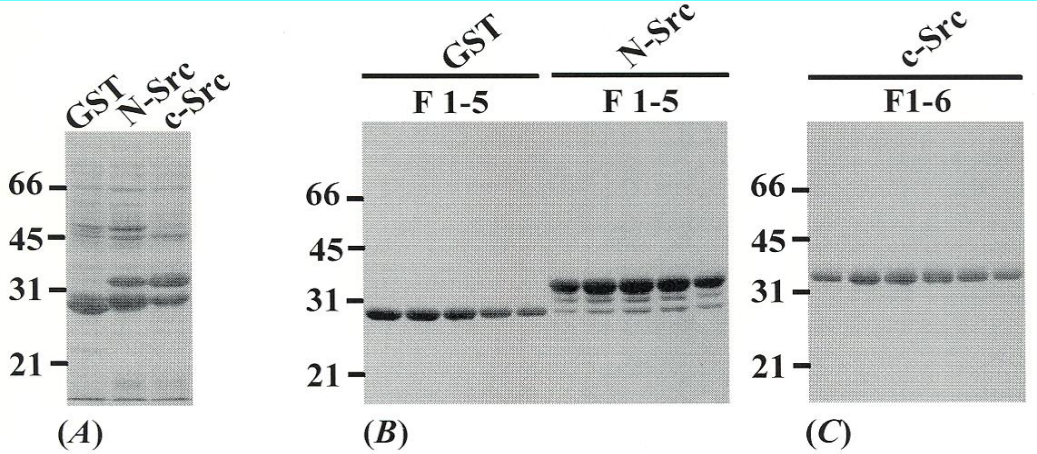
Fusion Protein Expression System

- **Fusion moiety: Highly-expressed**
  - **Glutathione-S-transferase (GST)**
  - **Histidine tag (His)**
- **Advantages:**
  - **Good translation initiation**
  - **Better stability of small proteins**
  - **Built in purification**
  - **Reliable & reproducible**
- **Limitations**
  - **Inclusion bodies**

GST/SH3 domain-containing Fusion proteins: 1



GST/SH3 domain-containing Fusion Proteins: 2



Rate-Limiting Factors

- **Plasmid-related:**
  - **Plasmid copy number**
  - **Transcriptional efficiency**
  - **Translational efficiency**
- **Host-related:**
  - **Genetic background**
  - **Growth requirement**
  - **Protease activity**

Gene Expression Protocol

- Suitable *E. coli* expression system
- Assemble the expression construct
- Transform a series of *E. coli*
- Screen transformants
- Confirmation: Target protein
- Optimize the expression yield

Troubleshooting - 1

- **Low yield:**
  - **Plasmid instability:**
    - **Alter growth conditions**
    - **Rec A$^-$ *E. coli* strains**
    - **Freshly-transformed colony for expression**
  - **Stretch of rare codons:**
    - **Replace with preferred codons**
  - **Protein degradation:**
    - **Protease-deficient mutant strains**
    - **Use fusion or secretory systems**

Troubleshooting - 2

- **Inclusion bodies:**
  - **Alter growth & induction conditions:**
    - **Lower growth temperature**
    - **Use different media**
    - **Reduce induction time**
    - **Use another host strain**

Conclusions

Successful Expression Results

- Visible band at the expected molecular weight
- Use of appropriate control(s)
- Functional, biochemical and immunological characterization of the recombinant protein

Thank You