Molecular Cloning:
Construction of a recombinant DNA

Anneal foreign DNA fragment to cloning vector and ligate
Molecular Cloning: Cloning Vectors

• Plasmids
Grand-daddy of plasmids

- **EcoRI**
- **BamHI**
- **PstI**
- **SalI**

- **Ampicillin resistance** ($amp^R$)
- **Tetracycline resistance** ($tet^R$)

**pBR322**

(4,361bp)

**Origin of replication** (ori)

**PvuII**
Molecular Cloning: Cloning Vectors

- **Plasmids**
  - Origin of replication, determines the number of copies per cell
  - Marker genes: ampicillin and tetracycline resistance genes
  - Unique restriction enzyme cut sites
Creation of a polylinker or multiple cloning site

![Diagram showing the creation of a polylinker or multiple cloning site.](image)
1. Plasmids
   - Origin of replication, determines the number of copies per cell
   - Marker genes: ampicillin and tetracycline resistance genes
   - Unique restriction enzyme cut sites
     • Polylinker of MCS
   - Small size
     • Limitation is ~15,000 bp
Entry of DNA into cells

- **Chemical Transformation**
  - Treat cells with calcium chloride
  - Heat shock

- **Electroporation**
  - High voltage transiently makes bacterial membrane permeable
Special examples of different plasmids

• Expression vectors
Special examples of different plasmids

A. Expression vectors
   – Strong promoter for transcription
   – Ribosome binding site
   – Transcription terminator
   – Some way of controlling gene – making it inducible
Fusion proteins

Tag expressed protein with another protein or a short peptide
Type of Tags

• Fluorescent proteins
  – One example is the green fluorescent protein or GFP
Type of Tags

• Fluorescent proteins
  – Examples is the green fluorescent protein or GFP

• Epitope tag
  – Short peptide sequence which has an antibody that recognizes it specifically
Express tagged protein in a cell.

Make cell extract.

Precipitate tagged protein with specific antibody.

Precipitate

Separate precipitated proteins.

Identify new proteins in precipitate (e.g., with mass spectrometry).

Size markers Pure tagged protein Precipitate
Type of Tags

- Fluorescent proteins
  - Examples is the green fluorescent protein or GFP
- Epitope tag
  - Short peptide sequence which has an antibody that recognizes it specifically
- Metal chelator
  - Can bind to Ni or Co chelated and immobilized
Special examples of different plasmids

B. Shuttle vectors
- Contains origins of replication from two different organisms
- Can be replicated in both
- Often used to shuttle plasmids from bacteria to yeast

C. Cosmids
- Plasmids containing at least one cos (cohesive-end site) of lambda phage
- Up to 44 kbps
Molecular Cloning: Cloning Vectors

2. Bacteriophages: example is lambda
   - 1/3 of genome (48.5 Kb) is non essential
   - DNA is packaged into phage particles
   - Can only fit 40 – 53 Kb of DNA
   - Have an in vitro packaging system
   - Highly efficient at transforming bacteria
   - Can clone up to 23 Kb of DNA
λ DNA

Cleave by restriction enzyme and separate the fragments

Not required for lytic infection

Remaining λ DNA contains genes required for infection but is too small to package

~15-kb foreign DNA fragment

Anneal and ligate

~36 kb

Infective λ phage containing foreign DNA fragment

Chimeric DNA

In vitro packaging
3. Bacterial Artificial Chromosomes (BAC)
   - 100 to 300 Kb in size
   - Have selectable markers
   - Stable origin of replication
   - Size of inserts is ~100 KB
   - Uses electroporation
Cloning sites (include lacZ)

F plasmid
par genes

 restriction
endonuclease

BAC vector

CmR
ori

Large foreign DNA fragment with appropriate sticky ends

DNA ligase

Recombinant BAC

 electroporation

selection of chloramphenicol-resistant cells

Agar containing chloramphenicol and substrate for β-galactosidase

Colonies with recombinant BACs are white.
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4. Yeast Artificial Chromosomes
Molecular Cloning: Cloning Vectors

4. Yeast Artificial Chromosomes (YAC)
   - ARS or origin of replication
   - Selectable markers
   - CEN or centromere sequence for proper segregation
   - Telomere sequences
   - Suitable for very large DNAs
A DNA library

- Collection of DNA clones
- Source for gene discovery
- Largest example is genomic library
- Other subsets would be such things as:
  - cDNA libraries
- Can scan these libraries by DNA hybridization
Colonies grown on master plate

Velvet pressed to master plate and transferred to nitrocellulose filter

DNA bound to filter

Anneal labeled probe, wash and dry

Radioactive probe hybridizes with its complementary DNA

Autoradiograph and compare with master plate

Autoradiograph film

Colonies detected by probe

Blackening identifies colonies containing the desired DNA