Recombinant DNA Technology
Key Methods

1. **Cutting DNA**
2. **Pasting DNA**
3. **Engineering Recombinant DNA**
4. **Making DNA from mRNA**
5. **Copying DNA**
6. **Determining nucleic acid length**
7. **Sequencing DNA**
8. **Probing to identify a gene of interest**

Recombinant DNA Technology
Key Concepts

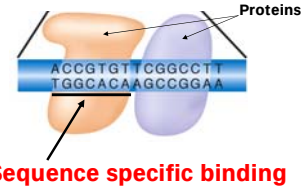
Two key properties of nucleic acids

ACGT
TGCA **Complementary**

5' ⟶ 3'
ACGT **Antiparallel**
TGCA
3' ⟵ 5'

Recombinant DNA Technology
Key Concepts

Property of Protein:nucleic acid interactions



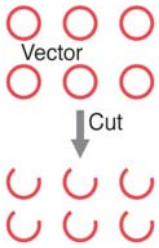
Sequence specific binding

Proteins bind to specific DNA sequences

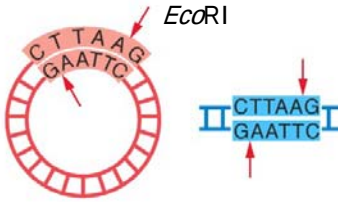
Recombinant DNA Technology
Cutting DNA

How to cut DNA

- Physical shearing
  - Random sites
- Enzymatic digesting
  - Endonuclease digestion: site specific
  - Restriction Endonucleases



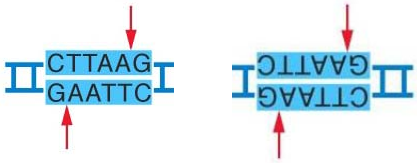
Restriction Endonuclease Digestion



Cutting DNA

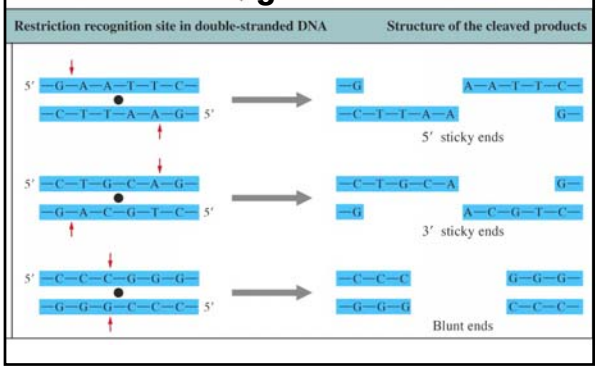
- Sequence specific
- enzymatic

Engineering Recombinant DNA



- Palindrome (Rotational symmetry)
- Cuts
  - Blunt/flush -Double stranded Blunt ends
  - Staggered -Single stranded "sticky ends"
    - 3' overhang
    - 5' overhang

Restriction Endonuclease Digestion



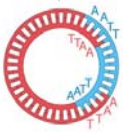
Recombinant DNA Technology
Cutting and Pasting DNA

Enzymology

Cutting

EcoRI

Restriction Endonuclease Digests DNA

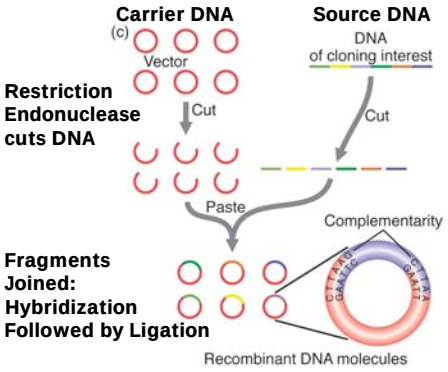


Pasting

DNA Ligase ligates DNA

Proteins (Enzymes) can cut and paste DNA

Engineering Recombinant DNA

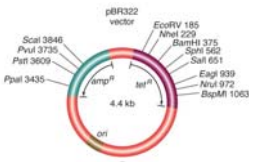


Engineering Recombinant DNA

Three Steps

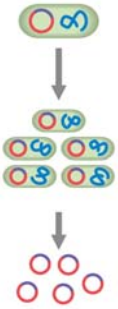
- 1. **Cut source and vector DNA**
  - **Restriction Endonuclease Digestion**
- 2. **Insert source fragment into vector**
  - **Hybridization / Ligation**
- 3. **Put recombinant vector into host**
  - **Transformation**

Engineering Recombinant DNA

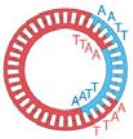


Vector DNA

- Origin of replication
- Capable of independent replication in a host
- Capable of incorporating DNA
- DNA sequence contains unique restriction site

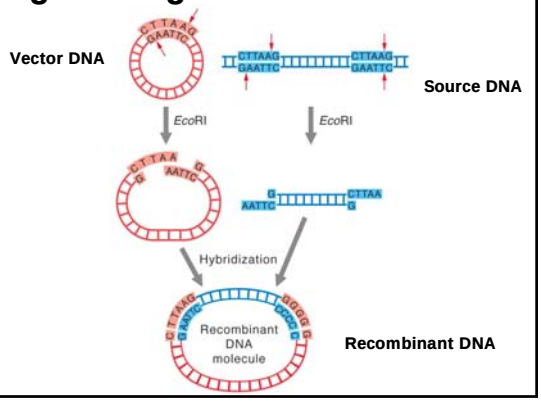


Insert source fragment into vector



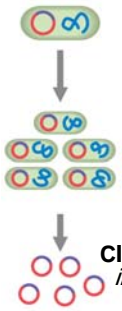
- **Hybridization (nonenzymatic)**
  - Sticky ends (complementary and antiparallel)
  - Salt and temperature
- **Ligation (enzymatic)**
  - DNA ligase – create phosphodiester bonds complete covalently joined sugar-phosphate backbones

Engineering Recombinant DNA

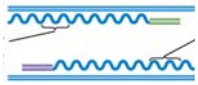


Recombinant DNA Technology

Copying DNA



Properties of DNA replication: polymerization



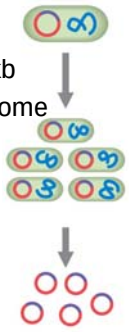
Cloning *in a host organism*

PCR Amplification *in a test tube*

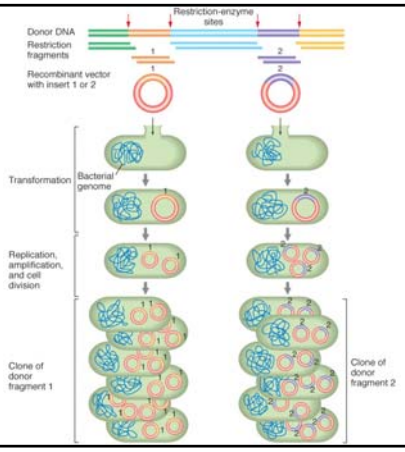
Proteins (Enzymes called polymerases) can make copies of DNA

Recombinant Molecules: Cloning

- In a host cell (Bacterial cells)
- Insert: range of sizes up to a few kb
- Cloning Vector: accessory chromosome
- Choice of vectors
- Choice of entry method into cell
- Replication in cell
- Recovery from cell



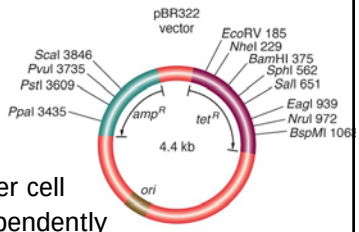
Recombinant Molecules: Cloning



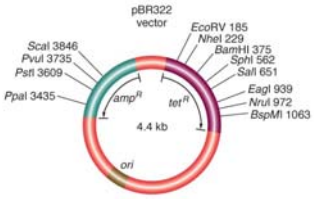
Recombinant Molecules: Cloning Vectors

Plasmids

- Small circular
- Many copies per cell
- Replicate independently
- Convenient restriction sites
- Unique (single cut) restriction sites



Recombinant Molecules: Cloning Vectors



- Means of identifying the recombinant vector
- Means of recovery of recombinant vector
- Choice depends on size of insert

Vectors

Bacteriophage vectors

- Single stranded
- Double stranded
- Size of insert limited
- Dispensible sequence can be replaced with insert sequence
- Headful packaging limits insert size