

## Recombinant DNA I

Basics of molecular cloning  
 Polymerase chain reaction  
 cDNA clones and screening

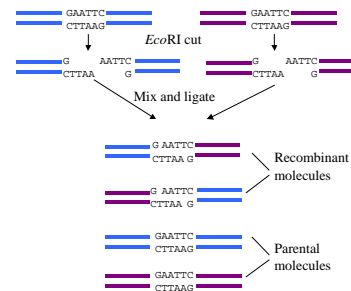
## Recombinant DNA Technology

- Utilizes microbiological selection and screening procedures to isolate a gene that represents as little as 1 part in a million of the genetic material in an organism.
- DNA from the organism of interest is divided into small pieces that are then placed into individual cells (usually bacterial).
- These can then be separated as individual colonies on plates, and they can be screened to find the gene of interest.
- This process is also called molecular cloning.

## DNA pieces are joined *in vitro* to form recombinant molecules

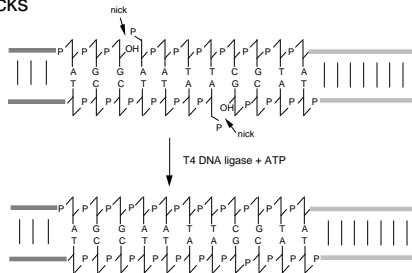
- Generate sticky ends on the DNA, e.g. with restriction endonucleases
- Tie DNA molecules from different sources together with **DNA ligase**

## Restriction endonucleases generate ends that facilitate mixing and matching

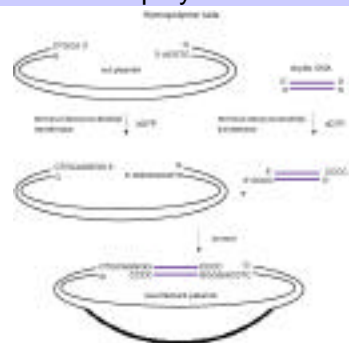


## DNA ligase covalently joins two DNA molecules

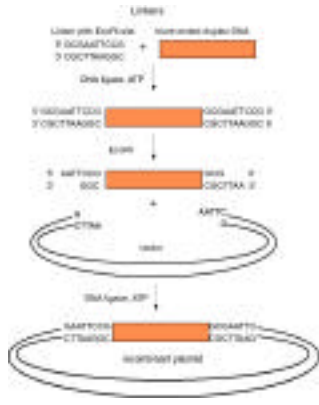
- Uses ATP or NADH to provide energy to seal nicks



## Alternate method to join DNA: homopolymer tails



Alternate method to join DNA: linkers



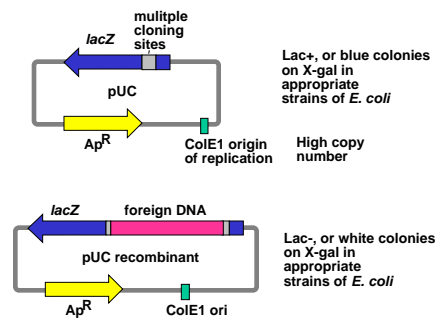
Introduction of recombinant DNA into living cells via **vectors**

- Autonomously replicating DNA molecules
  - (have an origin of replication)
- Selectable marker, such as drug resistance
  - (often a genetically engineered multiple cloning region with sites for several restriction enzymes)
- Insertion site for foreign DNA
  - (often a genetically engineered multiple cloning region with sites for several restriction enzymes)

Plasmid vectors

- Circular, extrachromosomal, autonomously replicating DNA molecules
- Frequently carry drug resistance genes
- Can be present in MANY copies in the cell

A common plasmid cloning vector: pUC



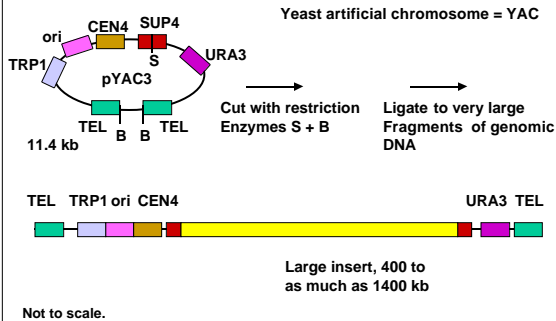
Transformation of *E. coli*

- *E. coli* does NOT have a natural system to take up DNA
- Treat with inorganic salts to destabilize cell wall and cell membrane
- During a brief heat shock, some of the bacteria takes up a plasmid molecule
- Can also use electroporation

Phage vectors

- More efficient introduction of DNA into bacteria
- Lambda phage and P1 phage can carry large fragments of DNA
  - 20 kb for lambda
  - 70 to 300 kb for P1
- M13 phage vectors can be used to generate single-stranded DNA

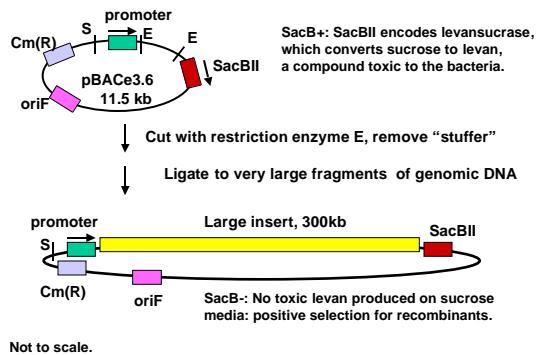
### YAC vectors for cloning large DNA inserts



### Bacterial artificial chromosomes

- Are derived from the fertility factor, or F-factor, of *E. coli*
- Can carry large inserts of foreign DNA, up to 300 kb
- Are low-copy number plasmids
- Are less prone to insert instability than YACs
- Have fewer chimeric inserts (more than one DNA fragment) than YACs
- Extensively used in genome projects

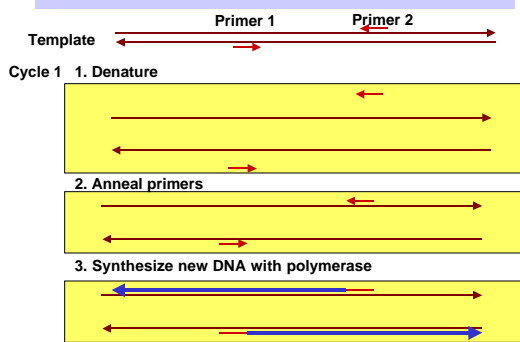
### BAC vectors for large DNA inserts



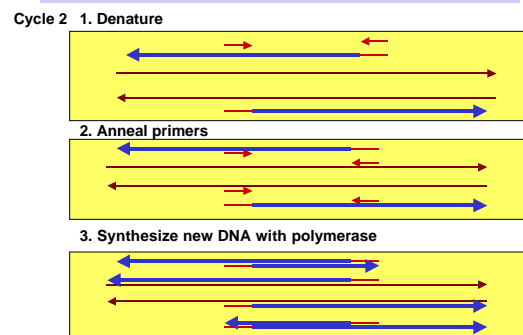
### PCR provides access to specific DNA segments

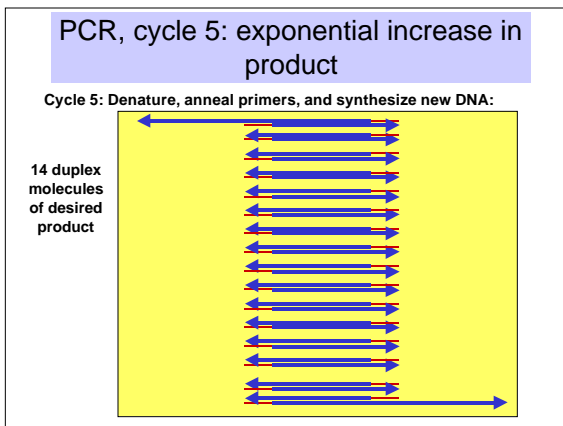
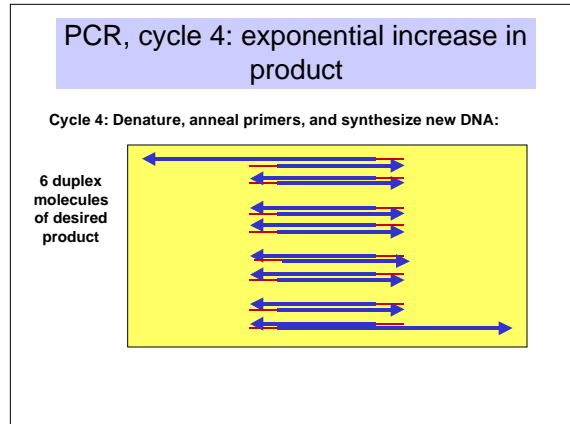
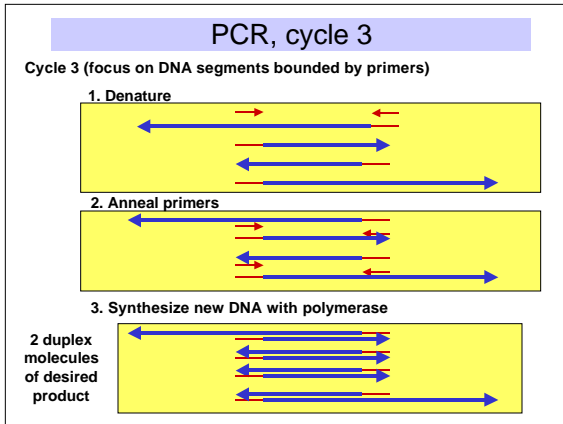
- **Polymerase Chain Reaction**
- Requires knowledge of the DNA sequence in the region of interest.
- As more sequence information becomes available, the uses of PCR expand.
- With appropriate primers, one can *amplify* the desired region from even miniscule amounts of DNA.
- Not limited by the distribution of restriction endonuclease cleavage sites.

### Polymerase chain reaction, cycle 1



### Polymerase chain reaction, cycle 2





- ### PCR: make large amounts of a particular sequence
- The number of molecules of the DNA fragment between the primers increases about 2-fold with each cycle.
  - For  $n =$  number of cycles, the amplification is approximately  $[2^{\exp(n-1)}]-2$ .
  - After 21 cycles, the fragment has been amplified about a million-fold.
  - E.g. a sample with 0.1 pg of the target fragment can be amplified to 0.1 microgram

- ### PCR is one of the most widely used molecular tools in biology
- Molecular genetics - obtain a specific DNA fragment
    - Test for function, expression, structure, etc.
  - Enzymology - place fragment encoding a particular region of a protein in an expression vector
  - Population genetics - examine polymorphisms in a population
  - Forensics - test whether suspect's DNA matches DNA extracted from evidence at crime scene
  - Etc, etc