CONCEPT: GENETIC CLONING

- The ability to _____ genes is the basis for all modern genetic advancements

1. Amplifying the DNA of interest to obtain many copies

- **Polymerase Chain Reaction (PCR)** is the main method used to amplify DNA

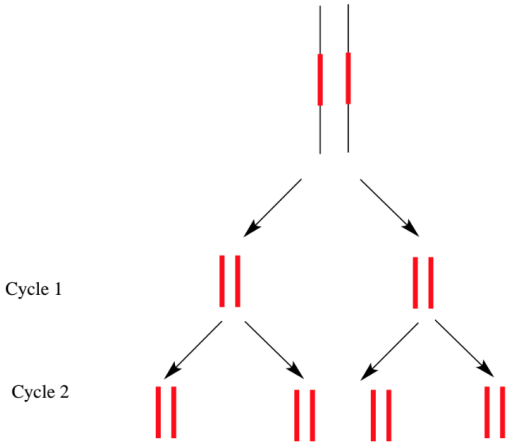
- Heat the DNA strands to a high temperature to separate the double helix

- Lower the temperature to **anneal** primers to the DNA sequence of interest

- Raise the temperature to allow the polymerase to find the primers and replicate the DNA

- Can be used to create and amplify cDNA from RNA

EXAMPLE:

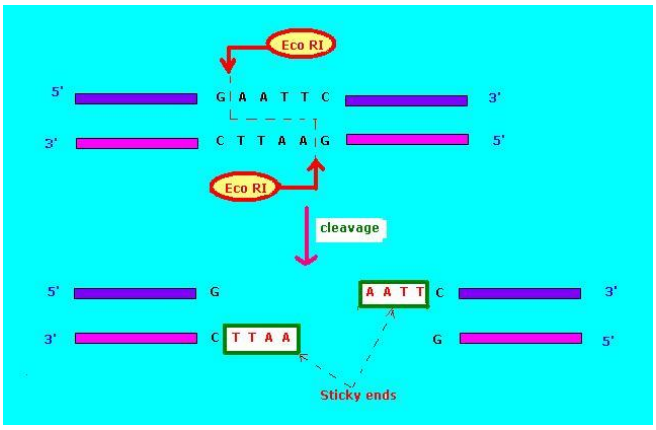


2. Cutting the DNA of interest into small _____

- **Restriction enzymes** are proteins used to chop DNA at specific sequences

- Can create **blunt ends** with no sequence overhangs or **stick ends** with sequence overhangs

EXAMPLE:



3. Pasting the DNA into a _____ (called **recombinant DNA**)

- **DNA ligase** is the enzyme that seals the DNA of interest into a plasmid

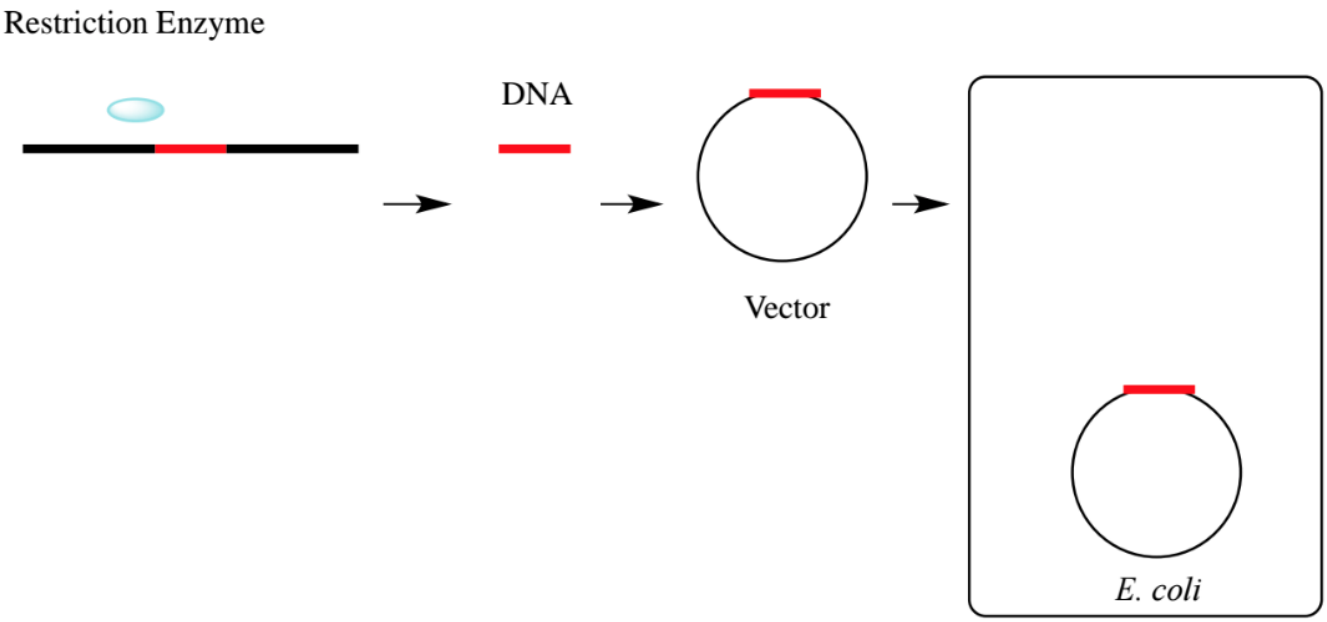
- Restriction enzymes create *sticky ends* that are overhangs to which the seal is made

- A **vector** is a bacterial plasmid in which the DNA sequence of interest is placed

- For large DNA inserts, **BACs** or **YACs** are used (bacterial or yeast artificial chromosomes)

- **T_i plasmids** are often used for plant cells

EXAMPLE:



4. The recombinant DNA is then placed in bacteria (*E. coli)* or another organism (ex: virus)

- A **transgenic organism** is created by adding a *transgene*

- A **transgene** is a new gene introduced into an organism

PRACTICE:

1. The purpose of polymerase chain reaction is to do what?
    a. Create RNA templates
    b. Create fluorescent probes
    c. Amplify a short DNA sequence
    d. Isolate proteins

2. Which of the following lists the steps of genetic cloning in the proper order?
    a. PCR → Restriction Enzyme Cutting → Ligate into a Vector → Placed into Organism
    b. Restriction Enzyme Cutting → PCR→ Ligate into a Vector → Placed into Organism
    c. Ligate into a Vector → PCR → Restriction Enzyme Cutting → Placed into Organism
    d. PCR → Ligate into a Vector → Restriction Enzyme Cutting → Placed into Organism