

Chapter 9
Recombinant DNA

I. Introduction to Biotechnology

- A. **Biotechnology** is the use of microorganisms, cells, or cell components to make a product.
- B. **Recombinant DNA (rDNA) technology** is the technology used to insert foreign DNA into an organism (commonly referred to as **genetic engineering**)
- C. Recombinant DNA technology was developed in the 1970s and 80s to express and amplify selected DNA in novel hosts.
- D. An overview of recombinant DNA procedures.
 - 1. Genes of interest are isolated from the environment.
 - 2. These genes are inserted into a genetic transport system, called **vector**, most commonly this is a plasmid when dealing with prokaryote rDNA technology.
 - 3. The modified vector (with inserted gene) is inserted into a carrier organism (commonly a bacterium), which is allow to multiply.
 - 4. Multiplied bacteria carrying the modified vector are called **clones**, since they derive from a single progenitor cell.
- E. Common uses for the clones
 - 1. Harvesting large numbers of the gene of interest for further manipulations.
 - 2. The cloned gene converts the phenotype of the modified host (i.e. BT toxin in modified plants).
 - 3. The protein is produced and harvested for other uses (i.e. insulin for the treatment of diabetes).

II. Tools of Biotechnology

- A. **Selection** – artificial selection is the selection of desirable traits in plants and animals. Microbiologists use the increased number of generations in bacteria to rapidly select for desired traits.
- B. **Mutation** – where as random mutations can result in unpredictable changes, microbiologist can make directed predicted mutations with the intent of creating desired reactions.
- C. **Restriction Enzymes** – a special class of DNA cutting enzymes that recognize specific DNA sequences.
- D. **Vectors** – DNA vehicles to carry inserted genes having a number of specific traits.
 - 1. **ori** – origin of replication to allow replication of the vector
 - 2. **Selection Sequence** – some phenotype encoded by the vector that can be selected for to determine the clones containing the vector (a common example is antibiotic resistance).
 - 3. **Cloning Site** – a small sequence stretch filled with specific restriction enzyme sites.

- E. **Polymerase Chain Reaction** – a process by which a specific DNA polymerase is used to amplify a specific sequence of DNA in a test tube.

III. Techniques of Genetic Modification

- A. Inserting Foreign DNA into Cells
 1. **Transformation** – as in the last chapter it can occur naturally in some species, but mainly it is accomplished through the use of chemicals to push DNA into cells.
 2. **Electroporation** – uses an electric current to force DNA into cells.
 3. **Protoplast fusion** – stripping away the cell wall of a cell and pushing DNA through the cell membrane with osmotic pressure.
- B. Obtaining DNA
 1. **Genetic Libraries** – random fragments of DNA from a specific organism.
 2. **Synthetic DNA** – gene sequences can be formulated in the lab and used.

IV. Applications

- A. **Genetic Screening** – the testing for the presence of specific sequences associated with a disease condition (i.e the “breast cancer” gene)
- B. **Forensic Microbiology** – tracking DNA fingerprints to determine the source of an infection.