Chapter 9 Biotechnology and Recombinant DNA

Summary Outline

9.1 Applications of **genetic engineering**
- A. **Protein production:** Bacteria can be engineered to efficiently produce pharmaceutical proteins, vaccines, and other proteins.
- B. **Providing a source of DNA for study**
  1. Segments of DNA can be cloned into *E. coli* and then used as a source for that sequence for study and further manipulation.
  2. Gene function and regulation can be more easily studied in *E. coli* because of the development of systems for the manipulation of DNA.
- C. **Altering organisms** to give them economically useful traits
  1. Transgenic plants can be made using a vector derived from the Ti plasmid of *Agrobacterium tumefaciens.*
  2. Examples include plants that resist pests and herbicides, plants with improved nutritional value, and plants that function as edible vaccines.

9.2 Applications of **nucleic acid hybridization**
- A. **DNA probes** are used to locate a specific nucleotide sequence in a DNA sample.
- B. **Colony blots** are used to identify colonies that contain the DNA sequence of interest.
- C. **Southern blots** are used to the size of the restriction fragments that contain a specific nucleotide sequence.
- D. **Fluorescence *in situ* hybridization (FISH)** – Specific nucleotide sequences within intact cells affixed to a microscope slide are detected by fluorescent-labeled probes.
- E. **Nucleotide array technologies** – Uses a **microarray** that is composed of tens or hundreds of thousands of oligonucleotides. Each oligonucleotide works in a manner similar to that of a probe.

9.3 Applications of **DNA sequencing**
- A. The **nucleotide sequence** of a gene can be used to determine the **amino acid sequence of the protein** for which it codes.
- B. Genetic alterations that occur in some disease can be identified.
- C. Evolutionary relationships can be studied.

9.4 Applications of polymerase **chain reaction**
- A. The **polymerase chain reaction (PCR)** is used to rapidly increase the amount of specific DNA segment in a sample.
- B. The **three-step amplification cycle**
  1. **Double-stranded DNA is denatured**.
  2. **Primers anneal to their complementary sequences**.
  3. **DNA is synthesized**, thus amplifying the target sequence.

9.5 Concerns regarding DNA technologies
- A. Ethical issues have been raised by the advances in **genomics**.
- B. Concerns exist about the introduction of allergens into a food product and adverse effects on the environment.

9.6 Techniques used in genetic engineering
- A. Genetically engineering bacteria
  1. C**loning into a population of *E. coli*** cells a set of DNA fragments that together make up the entire chromosome of the organism being studied resulting in a DNA library.

2. **Isolating DNA**
3. Using restriction enzymes to generate fragments of DNA
4. Generating a **recombinant DNA molecule**—To enable cloned DNA to replicate in a cell, it is attached to a piece of DNA called a vector, to form a recombinant molecule that is part vector and part cloned DNA.
5. **Introducing the recombinant DNA** into a new host using transformation or electroporation.

B. Genetically eukaryotic cells
1. **Vectors** used to clone DNA into eukaryotic cells are often specific for that cell type.
2. Introducing DNA into eukaryotic cells
a. **Ti plasmids** and viruses naturally carry DNA into eukaryotic cells.
b. Electroporation and a gene gun can be used to move DNA into eukaryotic cells.

9.7 Techniques used in nucleic acid hybridization

A. **Colony blotting** and **Southern blotting**
1. **DNA probes**—A probe, which is a labeled single-stranded piece of nucleic acid, is used to locate a specific nucleotide sequence in a DNA sample affixed to a nylon membrane.
2. **Colony blot**—Colonies are replica-plated on a nylon membrane; a DNA probe is then used to identify colonies that contain the sequence of interest.
3. **Southern blot—Gel electrophoresis** is used to separate DNA fragments according to size and the separated DNA is transferred in place to a nylon membrane. A DNA probe is then added to the membrane to locate specific nucleotide sequences.

B. **Fluorescence *in situ* hybridization** – Samples are treated to preserve the shape of cells, inactivate enzymes, and make the cells permeable.

C. **Nucleotide array technologies** – **Microarrays** are constructed and combined with the DNA to be studied.

9.8 Techniques used in DNA sequencing

A. **Dideoxy chain termination method**
1. A dideoxynucleotide is a nucleotide that lacks the 3'OH and functions as a chain terminator.
2. The sizes of fragments in a sequencing reaction indicate the positions of the terminating nucleotide base in the synthesized DNA strand.

B. **Automated DNA sequencing**
1. Each ddNTP(dideoxynucleotide) is labeled with a different color of fluorescent dye.
2. The reactions are done in a single tube and run on gel electrophoresis.
3. A laser detects the color of the band as it runs off the gel.

9.9 Techniques used in **polymerase chain reaction (PCR)**

A. The three step amplification cycle
1. **Double-stranded DNA is denatured**.
2. **Primers anneal to their complementary sequences**.
3. **DNA is synthesized**, thus amplifying the target sequence.

B. Generating a discrete-sized fragment
1. After three cycles of replication a discrete-sized fragment it amplified exponentially.
2. The size of the amplified fragment is dependent on the positions to which the primers anneal.

C. The selection of primer pairs determines which portion of the DNA is amplified.