

Study Outline Chapter 09

The Advent of Recombinant DNA Technology (pp. 242- 243)

- Closely related organisms can exchange genes in natural recombination.
- Genes can be transferred between unrelated species via laboratory manipulations called genetic engineering.
- Biotechnology includes all industrial applications of microorganisms, as well as industrial uses of genetically engineered cells.
- A DNA molecule used to carry a desired gene from one organism to another is called a vector.

An Overview of Recombinant DNA Procedures (p. 243)

- A desired gene is inserted into a DNA vector such as a plasmid or viral genome.
- The vector inserts the DNA into a new cell, which is grown to form a clone.
- Large quantities of the gene or the gene product can be harvested from the clone.

Biotechnology Tools and Techniques (pp. 243- 247)

- Prepackaged kits are available for many genetic engineering techniques.

Restriction Enzymes (p. 245)

- A restriction enzyme recognizes and cuts only one particular nucleotide sequence in DNA.
- Some restriction enzymes produce sticky ends, short stretches of single-stranded DNA at the ends of the DNA fragments.
- Fragments of DNA produced by the same restriction enzyme will spontaneously join by hydrogen bonding. DNA ligase can covalently link the DNA backbones.

Vectors (pp. 245- 247)

- Shuttle vectors are plasmids that can exist in several different species.
- A plasmid containing a new gene can be inserted into a cell by transformation.
- A virus containing a new gene can insert the gene into a cell.

Methods for Inserting Foreign DNA into Cells (pp. 247- 248)

- Cells can take up naked DNA by transformation. Chemical treatments are used to make cells that are not naturally transformed competent to take up DNA.
- Pores made in protoplasts and animal cells by electric current in the process of electroporation can provide entrance for new pieces of DNA.
- Protoplast fusion is the joining of cells whose cell walls have been removed.
- Foreign DNA can be introduced into plant cells by shooting DNA-coated particles into the cells.
- Foreign DNA can be injected into animal cells by using a fine glass micropipette.

Sources of DNA (pp. 245- 250)

- Gene libraries can be made by cutting up an entire genome with restriction enzymes and inserting the fragments into bacterial plasmids or phages.
- cDNA made from mRNA by reverse transcription can be cloned in gene libraries.

- Synthetic DNA can be made in vitro by a DNA synthesis machine.

Selecting a Clone (pp. 250- 252)

- Antibiotic-resistance markers on plasmid vectors are used to identify cells containing the engineered vector by direct selection.
- In blue-white screening, the vector contains the genes for ampR and b-galactosidase.
- The desired gene is inserted into the b-galactosidase gene site destroying the gene.
- Clones containing the recombinant vector will be resistant to ampicillin and unable to hydrolyze X-gal (white colonies). Clones containing the vector without the new gene will be blue. Clones lacking the vector will not grow.
- Clones containing foreign DNA can be tested for the desired gene product.
- A short piece of labeled DNA called a DNA probe can be used to identify clones carrying the desired gene.

Making a Gene Product (pp. 252- 253)

- E. coli is used to produce proteins by genetic engineering because it is easily grown and its genetics are well understood.
- Efforts must be made to ensure that E. coli's endotoxin does not contaminate a product intended for human use.
- To recover the product, E. coli must be lysed or the gene must be linked to a gene that produces a naturally secreted protein.
- Yeasts can be genetically engineered and are likely to continuously secrete the gene product.
- Mammalian cells can be engineered to produce proteins such as hormones for medical use.
- Plant cells can be engineered and used to produce plants with new properties.

Applications of Genetic Engineering (pp. 253- 261)

- Cloned DNA is used to produce products, study the cloned DNA, and alter the phenotype of an organism.

Genetically Engineered Products for Medical Therapy (pp. 253- 256)

- Synthetic genes linked to the b-galactosidase gene (lacZ) in a plasmid vector were inserted into E. coli, allowing E. coli to produce and secrete the two polypeptides used to make human insulin.
- Cells can be engineered to produce a pathogen's surface protein, which can be used as a subunit vaccine.
- Animal viruses can be engineered to carry a gene for a pathogen's surface protein. When the virus is used as a vaccine, the host develops an immunity to the pathogen.

Obtaining Information from DNA for Basic Research and Medical Applications (pp. 256- 260)

- Recombinant DNA techniques can be used to increase understanding of DNA, for genetic fingerprinting, and for gene therapy.

- DNA sequencing machines are used to determine the nucleotide base sequence in a gene.
- Southern blotting can be used to locate a gene in a cell.
- Genetic screening uses Southern blotting to look for mutations responsible for inherited diseases in humans.
- Gene therapy can be used to cure genetic diseases by replacing the defective or missing gene.
- Southern blotting is used in DNA fingerprinting to compare DNA recovered from a crime scene with that of a suspect.
- The polymerase chain reaction (PCR) is used to make multiple copies of a desired piece of DNA enzymatically.
- PCR can be used to increase the amounts of DNA in samples to detectable levels. This may allow sequencing of genes, the diagnosis of genetic diseases, or the detection of viruses.
- DNA probes can be used to quickly identify a pathogen in body tissue or food.

Agricultural Applications of Recombinant DNA Technology (pp. 260- 261)

- Cells from plants with desirable characteristics can be cloned to produce many identical cells. These cells can then be used to produce whole plants from which seeds can be harvested.
- Plant cells can be engineered by using the Ti plasmid vector. The tumor-producing T genes are replaced with desired genes, and the recombinant DNA is inserted into Agrobacterium. The bacterium naturally transforms its plant hosts.
- Rhizobium has been engineered for enhanced nitrogen fixation.
- Pseudomonas has been engineered to produce Bacillus thuringiensis toxin against insects.
- Bovine growth hormone is being produced by E. coli.

Safety Issues and the Ethics of Genetic Engineering (pp. 261- 263)

- Strict safety standards are used to avoid the accidental release of genetically engineered microorganisms.
- Some microbes used in genetic engineering have been altered so that they cannot survive outside of the laboratory.
- Microorganisms intended for use in the environment may be engineered to contain suicide genes so that the organisms do not persist in the environment.
- Genetic technology raises ethical questions such as: Should employers and insurance companies have access to a person's genetic records? Will some people be targeted for either breeding or sterilization? Will genetic counseling be available to everyone?

The Future of Genetic Engineering (p. 263)

- Genetic engineering techniques may provide new treatments for disease and new diagnostic tools.
- Genetic engineering techniques are being used to map the human genome through the Human Genome Project.

- This will provide tools for diagnosis and possibly the repair of genetic diseases.