CHAPTER 16 BIOTECHNOLOGY AND GENOMICS

Chapter Outline

16.1 Cloning of a Gene

A. Cloning
   1. **Cloning** is the production of identical copies through some asexual means.
   2. An underground stem or root sends up new shoots that are clones of the parent plant.
   3. Members of a bacterial colony on a petri dish are clones because they all came from division of the same cell.
   4. Human identical twins are clones; the original single embryo separate to become two individuals.
   5. **Gene cloning** is production of many identical copies of the same gene.
   6. If the inserted gene is replicated and expressed, we can recover the cloned gene or protein product.
   7. Cloned genes have many research purposes: determining the base sequence between normal and mutated genes, altering the phenotype, etc.
   8. Humans can be treated with **gene therapy**; alteration of other organisms forms **transgenic organisms.**

B. Recombinant DNA Technology
   1. **Recombinant DNA (rDNA)** contains DNA from two different sources.
   2. To make rDNA, technician selects a vector.
   3. A **vector** is a *plasmid* or a virus used to transfer foreign genetic material into a cell.
   4. A **plasmid** is a small accessory ring of DNA in the cytoplasm of bacteria.
   5. Plasmids were discovered in research on reproduction of intestinal bacteria *Escherichia coli.*
   6. Introduction of foreign DNA into vector DNA to produce rDNA requires two enzymes.
      a. **Restriction enzyme** is a bacterial enzyme that stops viral reproduction by cleaving viral DNA.
      b. The restriction enzyme is used to cut DNA at specific points during production of rDNA.
      c. It is called a restriction enzyme because it restricts growth of viruses but it acts a molecular scissors to cleave any piece of DNA at a specific site.
   7. Restriction enzymes cleave vector (plasmid) and foreign (human) DNA.
      a. Cleaving DNA makes DNA fragments ending in short single-stranded segments with "sticky ends."
      b. The "sticky ends" allow insertion of foreign DNA into vector DNA.
   8. The foreign gene is sealed into the vector DNA by **DNA ligase**.
      a. Treated cells take up plasmids, and then bacteria and plasmids reproduce.
      b. Eventually, there are many copies of the plasmid and many copies of the foreign gene.
      c. When DNA splicing is complete, an rDNA (recombinant DNA) molecule is formed.
   9. If the human gene is to express itself in a bacterium, the gene must be accompanied by the regulatory regions unique to bacteria and meet other requirements.
      a. The gene cannot contain introns because bacteria do not have introns.
      b. An enzyme called reverse transcriptase can be used to make a DNA copy of mRNA.
      c. This DNA molecule is called complementary DNA (cDNA) and does not contain introns.
      d. A laboratory DNA synthesizer can produce small pieces of DNA without introns.

C. The Polymerase Chain Reaction
   1. PCR can create millions of copies of a single gene or a specific piece of DNA in a test tube.
   2. PCR is very specific—the targeted DNA sequence can be less than one part in a million of the total DNA sample; therefore a single gene can be amplified using PCR.
   3. The polymerase chain reaction (PCR) uses the enzyme DNA polymerase to carry out multiple replications (a chain reaction) of target DNA.
   4. PCR automation is possible because heat-resistant DNA polymerase from *Thermus aquaticus,* which grows in hot springs, is an enzyme that withstands the temperature necessary to separate double-stranded DNA.

D. Analyzing DNA Segments
    1. Mitochondria DNA sequences in modern living populations can decipher the evolutionary history of human populations.
    2. **DNA fingerprinting** is the technique of using DNA fragment lengths, resulting from restriction enzyme cleavage and amplified by PCR, to identify particular individuals.
        a. DNA is treated with restriction enzymes to cut it into different sized fragments.
        b. During gel electrophoresis, fragments separate according to length, resulting in a pattern of bands.
        c. DNA fingerprinting can identify deceased individuals from skeletal remains, perpetrators of crimes from blood or semen samples, and genetic makeup of long-dead individuals or extinct organisms.
    3. PCR amplification and DNA analysis is used to:
        a. detect viral infections, genetic disorders, and cancer;
        b. determine the nucleotide sequence of human genes: the Human Genome Project; and
        c. associate samples with DNA of parents because it is inherited.

16.2 Biotechnology Products

A. Transgenic Organisms
    1. Genetically engineered organisms can produce biotechnology products.
    2. Organisms that have had a foreign gene inserted into them are **transgenic**.
B. Transgenic Bacteria
    1. Bacteria are grown in large vats called **bioreactors**.
        a. Foreign genes are inserted and the product is harvested.
        b. Products on the market include: insulin, hepatitis B vaccine, t-PA, and human growth hormone.
    2. Transgenic bacteria have been produced to protect and improve the health of plants.
        a. Frost-minus bacteria protect the vegetative parts of plants from frost damage.
        b. Root-colonizing bacteria receive genes from bacteria for insect toxin, protecting the roots.
        c. Bacteria that colonize corn roots can be endowed with genes for insect toxin.
    3. Transgenic bacteria can degrade substances.
        a. Bacteria selected for ability to degrade oil can be improved by genetic engineering.
        b. Bacteria can be bio-filters to prevent airborne chemical pollutants from being vented into the air.
        c. Bacteria can also remove sulfur from coal before it is burned and help clean up toxic dumps.
        d. Bacteria can also be given"suicide genes" that caused them to die after they have done their job.
    4. Transgenic bacteria can produce chemical products.
        a. We can manipulate genes coding for enzymes to catalyze synthesis of valuable chemicals.
        b. Phenylalanine used in aspartame sweetener can be grown by engineered bacteria.
    5. Transgenic bacteria process minerals.
        a. Many major mining companies already use bacteria to obtain various metals.
        b. Genetically engineered "bio-leaching" bacteria extract copper, uranium, and gold from low-grade ore.
C. Transgenic Plants
    1. Plant cells that have had the cell wall removed are called **protoplasts**.
    2. Electric current makes tiny holes in the plasma membrane through which genetic material enters.
    3. The protoplasts then go on to develop into mature plants.
    4. Foreign genes now give cotton, corn, and potato strains ability to produce an insect toxin and soybeans are now resistant to a common herbicide.
    5. Plants are being engineered to produce human proteins including hormones, clotting factors, and antibodies in their seeds; antibodies made by corn deliver radioisotopes to tumor cells and a soybean engineered antibody can treat genital herpes.
    6. Mouse-eared cress has been engineered to produce a biodegradable plastic in cell granules.
D. Transgenic Animals
    1. Animal use requires methods to insert genes into eggs of animals.
        a. It is possible to micro-inject foreign genes into eggs by hand.
        b. Vortex mixing places eggs in an agitator with DNA and silicon-carbide needles that make tiny holes through which the DNA can enter.
        c. Using this technique, many types of animal eggs have been injected with bovine growth hormone (bGH) to produce larger fishes, cows, pigs, rabbits, and sheep.

2. **Gene pharming** is the use of transgenic farm animals to produce pharmaceuticals; the product is obtainable from the milk of females.
    a. Genes for therapeutic proteins are inserted into animal's DNA; animal's milk produces proteins.
    b. Drugs obtained through gene pharming are planned for the treatment of cystic fibrosis, cancer, blood diseases, and other disorders.

E. Cloning Transgenic Animals
1. For many years, it was believed that adult vertebrate animals could not be cloned; the cloning of Dolly in 1997 demonstrated this can be cone.
2. Cloning of an adult vertebrate would require that all genes of an adult cell be turned on again.
3. Cloning of mammals involves injecting a 2n nucleus adult cell into an enucleated egg.
4. The cloned eggs begin development in vitro and are then returned to host mothers until the clones are born.

F Animal Organs as Biotechnology Products
1. It may be possible to use genetically engineered pigs to serve as a source of organs for human transplant.
2. Scientists are learning how to stimulate human cells to construct organs in the laboratory.

16.3 The Human Genome Project

A. The Human Genome Project had two goals: (1) to map the sequence of base pairs along our chromosomes and (2) to construct a map of the genes on all human chromosomes.
1. The first task is completed; it took 15 years to learn the sequence of the three billion base pairs along the length of our chromosomes.
2. The International Human Genome Sequencing Consortium was supported by public funds; Celera Genomics was funded by pharmaceutical industry.
3. There is little difference between the sequence of our bases and other organisms whose DNA sequences are known.
4. We share a large number of genes with simpler organisms; perhaps our uniqueness is due to regulation of these genes.

B. The Genetic Map
1. A genetic map will locate each gene along each chromosome.
2. With the base map completed, the chromosomal genetic map should be completed faster.
3. The total number of human genes appears to be far lower than expected–perhaps only 30,000.
4. With a roundworm possessing 20,000 genes, either more genes are yet to be found or each gene could code for three proteins by sing different combinations of exons.
5. A genetic map of a chromosome could help tailor medical treatments to an individual.
6. Gene therapy genes could be inserted into an egg before it is fertilized.
7. Such potentials raise many ethical issues.

16.4 Gene Therapy

A. Gene Therapy Inserts Healthy Genes
1. This includes procedures to give patient healthy genes to make up for a faulty gene.
2. Gene therapy also includes the use of genes to treat genetic disorders and various human illnesses.
3. There are ex vivo (outside body) and in vivo (inside body) methods of gene therapy.

B. Ex Vivo Gene Therapy
1. Children with severe combined immunodeficiency (SCID) underwent ex vivo gene therapy.
    a. Lacking the enzyme ADA involved in maturation of T and B cells, they faced life-threatening infections.
    b. Bone marrow stem cells are removed, infected with a retrovirus that carries a normal gene for the enzyme ADA, and returned.
    c. Use of bone marrow stem cells allows them to divide and produce more cells with same genes.
    d. Patients who undergo this procedure show significant improvement.
2. Gene therapy trials include treatment of familial hypercholesterolemia where liver cells lack a receptor for removing cholesterol from blood.
    a. High levels of blood cholesterol make the patient subject to fatal heart attacks when young.
    b. A small portion of the liver is surgically removed and infected with retrovirus with normal gene for receptor.
    c. This has lowered cholesterol levels following the procedure.

C. In Vivo Gene Therapy
    1. Cystic fibrosis patients lack a gene for trans-membrane chloride ion carriers; patients die from respiratory tract infections.
        a. Liposomes, microscopic vesicles that form when lipoproteins are in solution, are coated with healthy cystic fibrosis genes and sprayed into a patient's nostrils.
        b. Various methods of delivery are being tested for effectiveness.
    2. A gene for vascular endothelial growth factor (VEGF) can be injected alone or within a virus into the heart to stimulate branching of coronary blood vessels.
    3. Another strategy is to make cancer cells more vulnerable, and normal cells more resistant, to chemotherapy.
    4. Injecting a retrovirus containing a normal *p*53 gene–that promotes apoptosis–into tumors may stop the growth of tumors.