Biotechnology

- Recombinant DNA Technology
- Gene Sequencing (Human Genome Project)
- Cloning
- Stem Cell Research
- Gene Therapy
- DNA Fingerprinting (and other Forensics

16.1 DNA Cloning

16.1 Cloning of a Gene

1. **Cloning** is the production of identical copies
2. An underground stem sends up new shoots that are clones
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.**

***More on cloning later.**

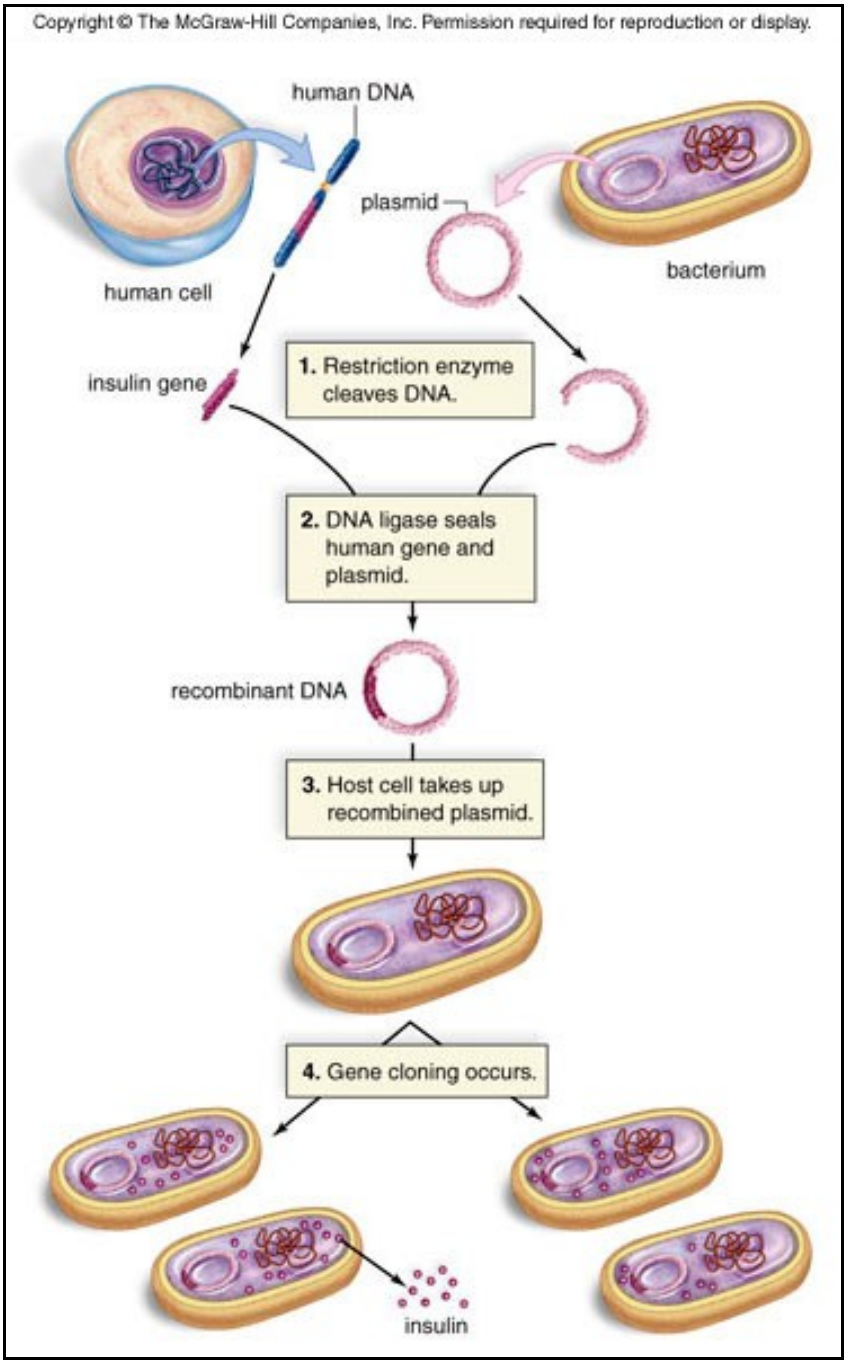
B. Recombinant DNA Technology

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.



C. The Polymerase Chain Reaction

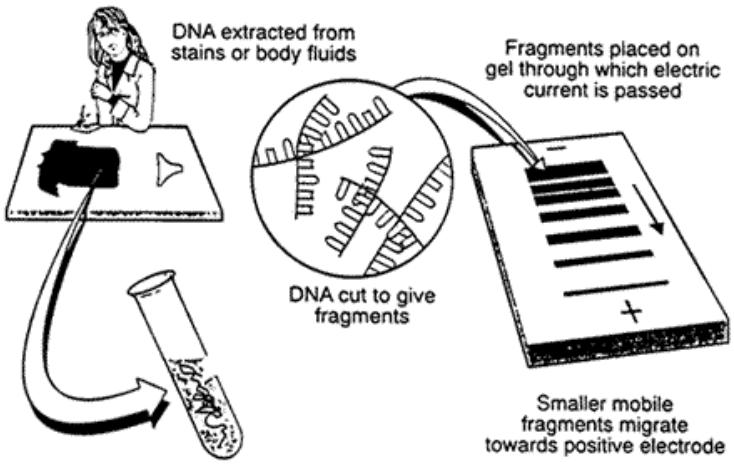
1. PCR can create millions of copies of a single gene or a specific piece of DNA in a test tube.
2. PCR is very specific—the targeted DNA sequence can be less than one part in a million of the total DNA sample; therefore a single gene can be amplified using PCR.
3. The polymerase chain reaction (PCR) 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 doublestranded 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 - Organisms that have had a foreign gene inserted into them

B. Transgenic Bacteria

1. Bacteria are grown in large vats called **bioreactors**.
a. Foreign genes are inserted and the product is harvested. (insulin, hepatitis B vaccine, t-PA, and human growth hormone)
2. Transgenic bacteria have been produced to protect and improve the health of plants (frost resistance, pesticides)
3. Transgenic bacteria can degrade substances (oil-eating bacteria, bio-filters)
4. Transgenic bacteria can produce chemical products. (phenylalanine for aspartame sweetener)

C. Transgenic Plants
1. Foreign genes now give cotton, corn, and potato strains ability to produce an insect toxin
2. 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.

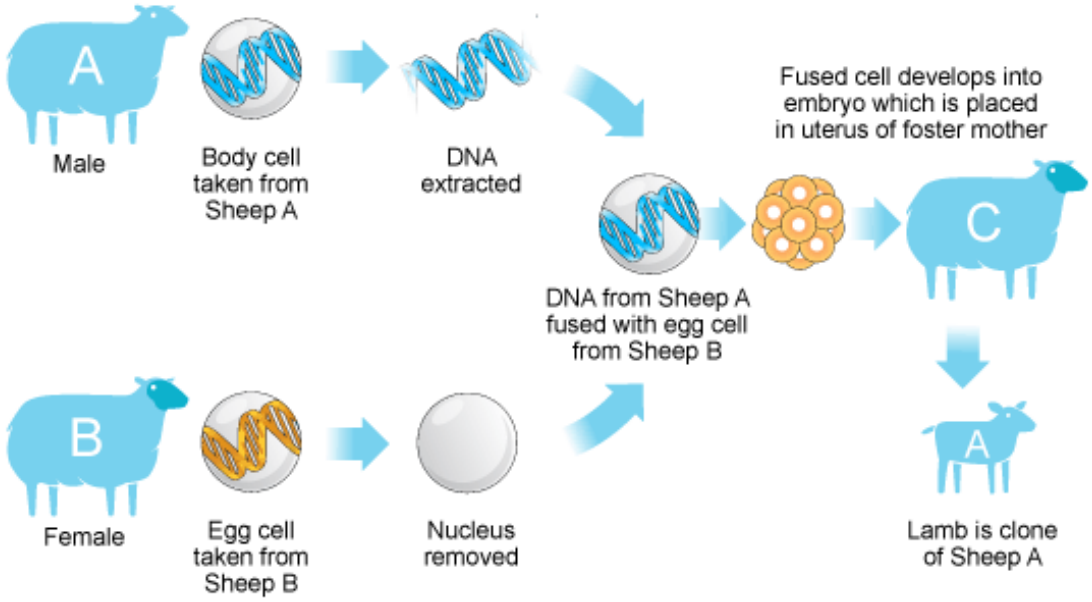
D. Transgenic Animals
1. Animal use requires methods to insert genes into eggs of animals.

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 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; 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.
5. **Somatic Cell Nuclear Transfer** – cloning from adult cells (Dolly)



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.

Scenario 1: Melissa is a happy 5 year old who is loved by her family. She becomes ill and is diagnosed with childhood leukemia. A desperate search ensues to find a bone marrow donor whose type matches Melissa. After a year of searching, Melissa's outlook is grim. Her family decides to clone Melissa so that her clone could be the bone marrow donor. Do you think this is a god idea? Why or why not.

Scenario 2: A well-loved horse named Barbero breaks his leg in a race. Many people were praying for his well being and thousands of dollars were spent trying to get him to recover. Mail and flowers poured into the animal hospital and stable where Barbero lived. Alas, after a year of poor recovery, the decision was made to euthanize Barbero. The owners save sample of his DNA so that Barbero can be cloned. Do you think they should clone him? Why or why not.