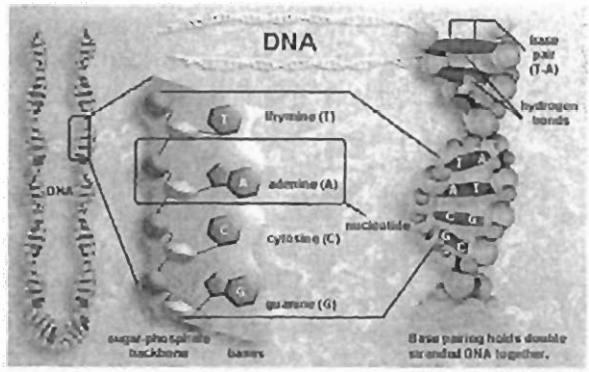
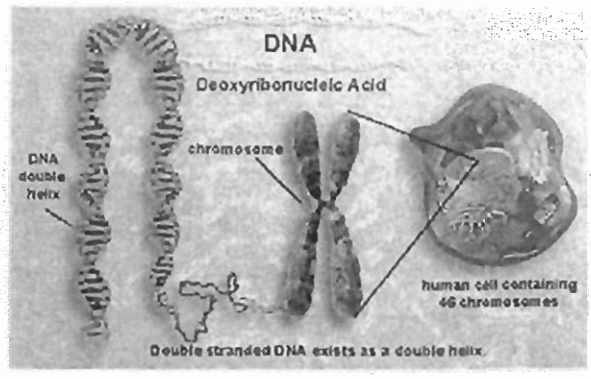
Study Guide #1
DNA OVERVIEW

DNA, or deoxyribonucleic acid, is the material within a cell that stores and encodes genetic information. DNA allows this information to be passed on from one generation to the next.



The molecular structure of DNA is the same for all species on the planet. DNA consists of two long chains of molecules linked together in a pattern called a double helix, which resembles a ladder twisted around its long axis.

The long chains of the double helix are comprised of structural units known as **nucleotides**. Nucleotides consist of a sugar, a phosphate group and a nitrogenous base all linked together. There are four nitrogenous bases: adenine, **thymine**, **cytosine** and **guanine**. The sugar and phosphate make up the backbone of the double helix. Hydrogen bonding between the nitrogenous bases holds the two chains of the double helix together, like the "rungs" of a ladder. Pairing between the four bases, known as base pairing, always occurs in a particular pattern. Adenine always bonds with thymine, and guanine always bonds with cytosine. The sequence of nucleotides and amount of DNA varies from species to species, but the basic building blocks remain the same.

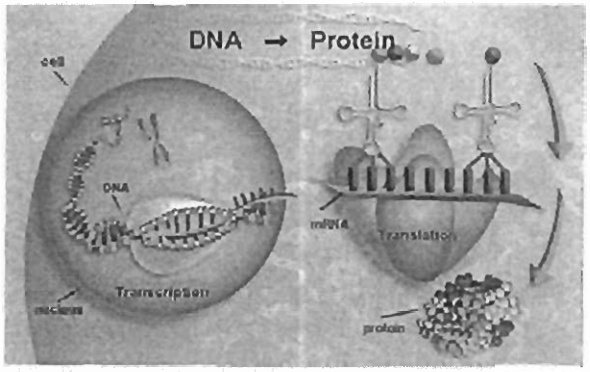


The long strands of the DNA double helix are stored within cells as tightly wound structures called **chromosomes**. Specific nucleotide sequences make up what are known as **genes**. Genes code for the production of specific proteins. Some proteins have observable effects, such as hair or eye color, while others, such as blood proteins, are less obvious.

Genes serve as the blueprints for proteins. There are many types of proteins found in every living cell. Proteins can function as **enzymes**, which are molecules that greatly speed up the rate of chemical reactions. They can also serve as **antibodies**, which attach to foreign objects to help fight infection. Proteins can also be cellular transporters like **hemoglobin**, which serves to carry oxygen in the blood. In addition, proteins can serve as structural materials, such as the long molecules of keratin which make up human hair and nails.

When genes are activated so that their coded genetic information serves as a template for protein production, the genes are said to be expressed. In a process

known as **transcription**, a copy of a single strand of DNA is created and exported from the nucleus of the cell. This copy of the single DNA strand is called **messenger ribonucleic acid** or **mRNA**. This mRNA is converted into a protein or "read" during a process called **translation**. During this process, **amino acids**, the building blocks of proteins, are linked together in a sequence specified by the mRNA. These amino acids are eventually folded into the protein coded by the gene.

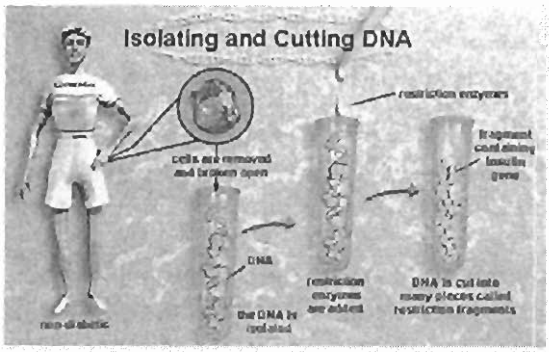


Genes for specific proteins show variation within a **population** of organisms. Through the process of **selective breeding**, humans take advantage of this natural variation by breeding organisms to obtain particular traits. For example, many of our food crops have been bred to combine the naturally occurring disease resistance of one strain with naturally occurring heavy fruit production of another strain.

While selective breeding is limited to using only naturally occurring traits, there is a way in which entirely new traits can arise. **Mutation** is a change in the DNA sequence of the genetic code. Mutations can occur naturally, or they can be brought about by exposing organisms to radiation or ultraviolet light. Most mutations are not beneficial, because they change a functional DNA sequence. Occasionally though, mutation can give rise to a new or beneficial trait.

Study Guide #2
GENETIC ENGINEERING OVERVIEW

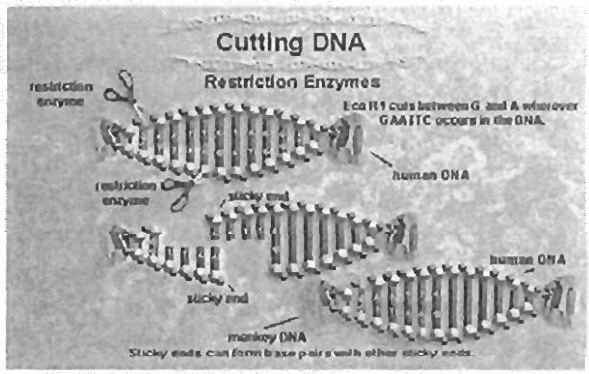
With advancements in our understanding of DNA has come the ability to alter traits at the level of the gene. Since DNA is the same for all organisms on earth, scientists are able to transfer genes for specific proteins or traits between entirely different organisms. DNA transferred from one organism to another is referred to as **foreign DNA**, because the receiving organism did not originally possess those genes. Sometimes foreign genes will be expressed, meaning that the proteins or traits coded for by the gene are actually produced.



If changes to DNA are made in reproductive cells or early embryos, these changes could be inherited by future generations of the organism. However, if changes are made to somatic, or non-reproductive, cells these changes will not be passed on.

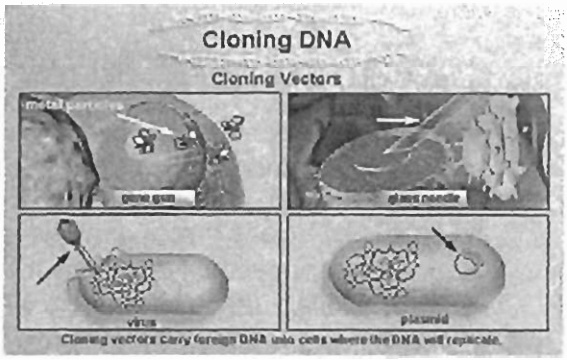
There are several methods for transferring genes between organisms, but the basic steps are usually the same. Cells from the organism are broken open, and the entire cellular DNA is removed. This DNA is then cut into thousands or even millions of tiny fragments by **restriction enzymes**. These powerful molecules function at the molecular level to break DNA chains into tiny pieces called **restriction fragments**, one of which may contain the desired genes.

Restriction enzymes cut DNA at specific nucleotide sequences. For example, the restriction enzyme Eco R1 cuts DNA between guanine (G) and adenine (A) wherever the nucleotide sequence G-A-A-T-T-C occurs. The restriction enzyme produces a staggered cut, which forms two single-stranded regions, called sticky ends, on each restriction fragment . Sticky ends are named as such because they enable restriction fragments cut by the same restriction enzyme to bond, or stick together.

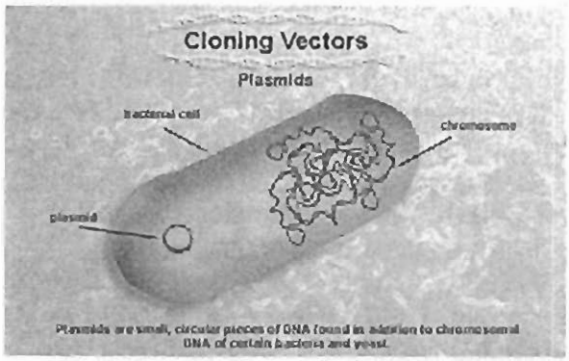


In order to give the scientists enough material to work with, many copies of the DNA fragments are made. Producing copies of DNA is called **cloning**. Clones are genetically identical to the fragments from which they were produced. The process of cloning restriction fragments prior to isolating the desired gene is commonly referred to as the **shotgun approach**.

Clones are usually produced by inserting restriction fragments into the DNA of cells where it will naturally be replicated along with the rest of the cell's DNA during cellular division. In order to place foreign DNA into a cell, specific devices known as cloning **vectors** are used. One type of vector is called a "**gene gun**". The gene gun shoots microscopic metal particles coated with DNA directly into the cells. Another type of vector is a tiny glass needle, which is used to inject DNA into the nucleus of a cell. Finally, **viruses** and small circular pieces of DNA called **plasmids** can be used to transport foreign DNA into a cell where it will replicate.

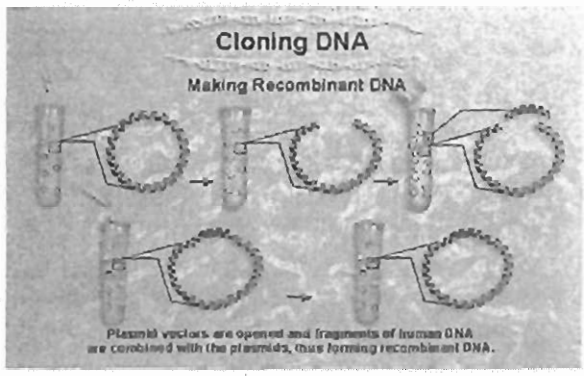


Viruses are composed of either DNA or RNA surrounded by a protein coat. When viruses are used as cloning vectors, the viral DNA is removed, and replaced with the foreign DNA which genetic engineers wish to insert into a cell.

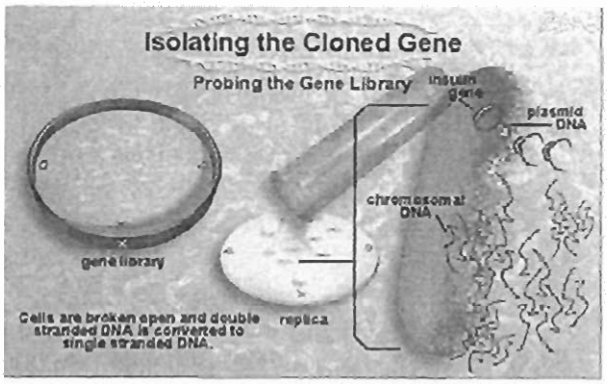


Plasmids are small, circular pieces of DNA found in some bacteria and yeast cells. They have several features that make them ideally suited for use as vectors. Their small size allows them to be easily separated from chromosomal DNA by centrifugation. The small size also means there is usually only one site where a particular restriction enzyme will cut the plasmid, resulting in linear DNA which can re-close to the original circular plasmid shape when combined with a foreign DNA fragment. In addition, plasmids are naturally transferred from bacterium to bacterium. Finally, some plasmids contain genes that allow bacteria to survive in the presence of an antibiotic. Knowing this, researchers can use antibiotic-resistance plasmids to their advantage. It is easy to separate cells containing an antibiotic resistant plasmid from other cells simply by adding an antibiotic to kill the cells that don't contain the plasmid.

Once DNA has been cut into restriction fragments, it's mixed with plasmid vectors that have been cut by the same restriction enzyme. The sticky ends of the foreign DNA combine with the sticky ends of the plasmid vector. Another enzyme called **ligase** is then added. Ligase acts as a molecular glue, and attaches the sugar phosphate backbone of the foreign DNA to that of the plasmid vector. Thus, the plasmid vector is restored to its original



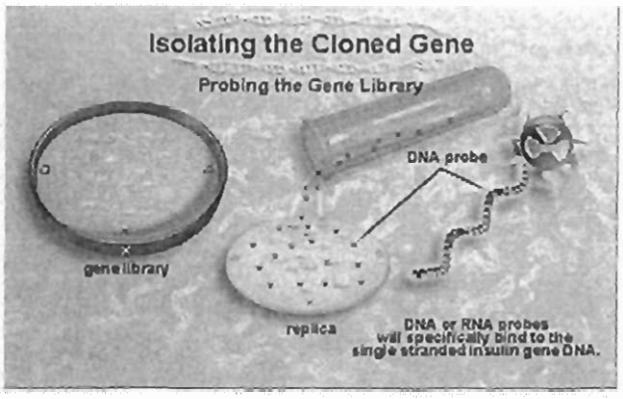
circular shape, with the addition of a fragment of foreign DNA. When the DNA from two different organisms is recombined in this fashion, it is referred to as **recombinant DNA**.



Once recombinant plasmids have been produced, they are ready for insertion into bacterial cells. The process of introducing new DNA into a cell is called **transformation**. In order to facilitate the process, chemicals are often used to weaken the cell membranes of the bacteria. Likewise, pulses of electricity can be used to punch temporary holes through the cell membrane to allow the passage of plasmids. This process is called **electroporation**.

If recombinant plasmids contain a gene for resistance to an antibiotic, isolation of bacteria transformed with a plasmid is a relatively simple process. If an antibiotic is introduced to the bacteria, only those with the recombinant plasmid containing antibiotic resistant genes will survive.

After bacteria have been transformed with recombinant plasmids, they are allowed to grow until each bacterial cell produces a colony consisting of millions of genetically identical clones. At this point, there are many different colonies corresponding to the many original fragments of foreign DNA. Together, the entire group is referred to as a **gene library**. Some of these colonies contain the gene fragments a genetic engineer wishes to isolate, but many do not. At this point, the transformed bacterial colony containing the desired gene still needs to be isolated from the other bacterial colonies.



Once the colonies containing all the various DNA fragments have grown, scientists isolate the colony containing the desired gene through the use of RNA probes, or single-stranded DNA probes which bind to the DNA of the desired gene by forming base pairs with it. Probes that will pair with specific genes can be isolated or created in the laboratory through intricate techniques. Radioactive molecules that expose x-ray film are then attached to the probes. Once the probes bind to DNA from cells of various colonies, they are exposed to x-ray film. The radioactive particles create dark spots on the film, allowing researchers to determine where the probes attached.

In order to examine the various colonies in this fashion without damaging the living bacteria, a copy of the colonies is made, usually by touching a piece of filter paper to

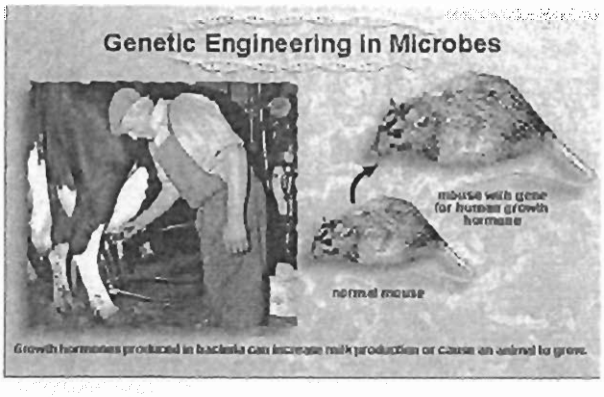
tops of the colonies on the petri dish. Cells from each colony adhere to the filter paper, which is then treated with chemicals that break open the cells and convert the double-stranded DNA into two single strands. Radioactive DNA or RNA probes are then added and bind to the single-stranded DNA of the gene the scientists are attempting to isolate. Unbound probes are washed off before the x-ray film is placed over the filter paper so that only areas with bound probes will show up on the film. By comparing the positions of the dark spots with the positions of the various bacterial colonies, the colony containing the desired gene can be determined.

If bacteria used to make copies of a desired gene also express that gene, or produce the protein for which the gene codes, scientists can use the gene product to identify colonies containing the gene. Antibodies that attach to a particular protein are used to identify colonies producing the protein coded for by the desired gene. Radioactive molecules are attached to the antibodies as with the DNA or RNA probes mentioned earlier, or in an easier method, fluorescent molecules are attached to the antibodies. The fluorescent molecules will shine when exposed to ultraviolet light, thus they can identify colonies that produce the desired protein.

After transformed bacteria containing the desired gene have been isolated from the rest of the bacterial colonies in the gene library, they are allowed to grow. This provides a steady source of the desired genes, and if the bacteria express the gene, a steady source of the proteins coded for by the gene as well. Often the genes are transferred to other bacteria that express genes at a high rate. Another method of increasing production involves using plasmids that make multiple copies of themselves in each cell.

Study Guide #3
GENETIC ENGINEERING IN MICROBES

In 1985, bacteria were genetically engineered to produce human growth hormone. Approximately 1 in 100,000 people suffers from a genetic defect called **pituitary dwarfism**. Before bacteria were engineered to produce the growth hormone, it was extracted from the pituitary glands of corpses. This former procedure had several disadvantages: many corpses were required to treat a single patient, and some patients received pituitary extract contaminated with viruses.



Bacteria have also been engineered to produce growth hormones for other species. For example, **bovine growth hormone** was approved for used in dairy cows in the United States and other countries in 1994. In dairy cows injected bi-weekly with bovine growth hormone, milk production can be increased by as much as twenty percent. Growth hormones have also been found to work on species other than those from which they are isolated. For example, mice which have been engineered with the gene for the human growth hormone grow to roughly twice their normal size.

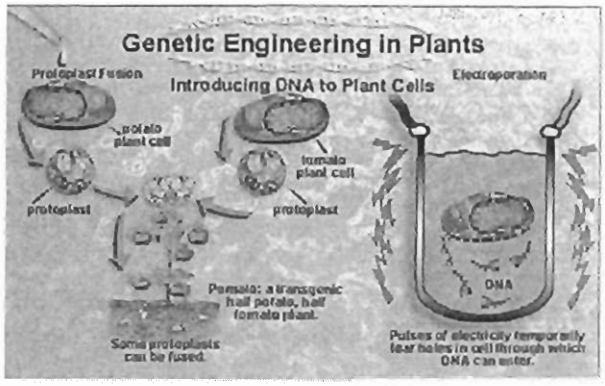
Another example of genetic engineering in microbes is a waterproof glue used in certain dental and medical procedures. The genes for the production of this glue were isolated from mussels, which use it to attach themselves to rocks and piers. When the genes were transferred to bacteria, they produced it in large enough amounts for medical use.

Genetically engineered microbes are also used in cheese production. Originally, cheese was produced by curdling milk with **rennin**, an enzyme isolated from the stomach lining of calves. The gene that codes for the rennin enzyme was isolated and transferred to yeast. In 1990, this enzyme was approved for use in dairy products.

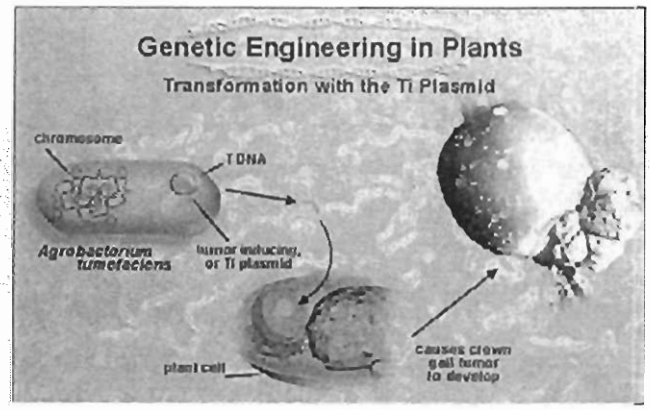
Study Guide #4
GENETIC ENGINEERING IN PLANTS

As the world's population increases, genetic engineering of plant crops has become increasingly important. Plants are engineered through selective breeding and gene transfer for better yield, greater nutritional value, and greater resistance to insect pests and harsh growing conditions such as those found in arid deserts or marsh areas with high salt concentrations.

Genetic engineering in plants poses its own difficulties. Unlike animal cells, plant cells are surrounded by a rigid cell wall. Scientists had to figure out how to get DNA through the cell wall and into the cell. This was accomplished by using chemicals to break down the cell wall, creating what are known as **protoplasts**, plant cells which have lost their cell wall and taken on a spherical shape. Protoplasts can be transformed with foreign DNA. In addition, protoplasts can sometimes be fused directly as a method of creating **transgenic** organisms, which like all recombinant organisms, contain genes from two different organisms.



DNA can also be transported through plant cell walls by **electroporation**. This, as you will recall, is the process of using pulses of electricity to punch small, temporary holes in a cell through which DNA can be inserted.



Another method for transporting DNA into plant cells involves using a bacterium called *Agrobacterium tumefaciens*, which contains a plasmid called the **Ti**, or Tumor inducing plasmid. This plasmid naturally transfers part of its genetic material to plants. This genetic material, called T DNA, actually enters the nucleus of plant cells and becomes incorporated into the plant's DNA. In nature, the T DNA codes for proteins that produce a type of plant tumor called a crown gall. However, scientists can remove the T DNA responsible for tumor production and replace it with genes they wish to introduce into a plant.

An example of the use of the Ti plasmid in genetic engineering of plants includes the insecticidal bacterium, ***Bacillus thurigiensis***. This bacterium produces crystal-like

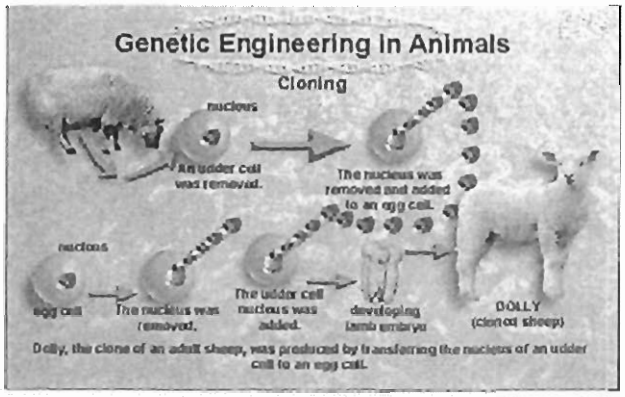
proteins, which damage the digestive system of insect larvae. Since the early 1960's, *Bacillus thurigiensis* bacteria have been applied to crops in the form of a dust, however it must be applied to the crops several times per season. In the mid 1990's, scientists succeeded in using the Ti plasmid to transfer the insecticide-producing gene from the *Bacillus thurigiensis* to cells isolated from various crop plants. These cells were eventually grown into entire plants containing effective levels of the insecticide.

Genetic engineering techniques have also been used to produce longer-lasting tomatoes as well as strawberry plants that are resistant to frost. There is also hope that genetic engineering will eventually allow crops to fix their own nitrogen from the atmosphere as do the bacteria contained in the root nodules of legumes such as beans. Unfortunately, many different genes are involved in nitrogen fixation, so it will be difficult to isolate and transfer them all into a plant while still maintaining their proper function.

Study Guide #5
GENETIC ENGINEERING IN ANIMALS

Animal cells lack plasmids. As a result, animals tend to be more difficult than bacteria or yeast to genetically engineer. Foreign DNA is usually inserted into animal cells via specialized viruses and glass needles, as mentioned earlier. Most research into the genetic engineering of animals has thus far focused on producing larger livestock. However, as our knowledge of genetic manipulation expands, and as new techniques are developed, new uses for genetically engineered animals are arising.

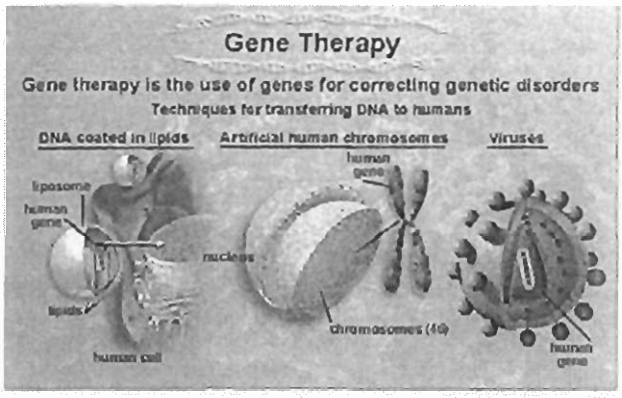
Several types of animals have been engineered to produce products for use in the human body. For example, pigs have been engineered to produce organs coated in human proteins. The organs are used for transplants, and lessen the risk of the body rejecting the foreign organ. Sheep have also been engineered to produce milk with proteins used to treat genetic forms of hemophilia and emphysema



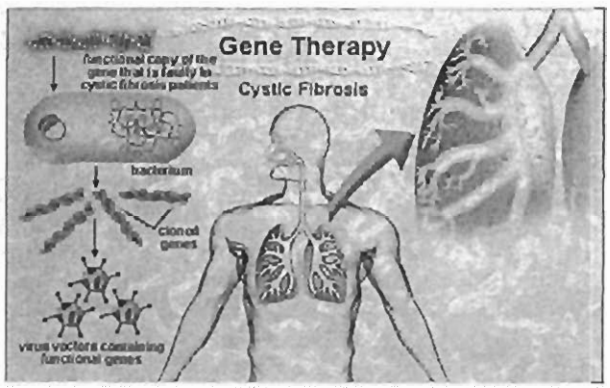
One of the more interesting developments in the genetic engineering of animals involves animal cloning. Animals were first cloned by removing the nucleus of an egg and replacing it with the nucleus of a cell taken from a developing embryo. However, in 1997 a major breakthrough in cloning was made when a clone was produced from a cell taken from a mature sheep instead of a developing embryo. The same general technique was employed, but the nucleus used to replace the nucleus of the egg came from an udder cell of a mature ewe. The result was the now famous cloned sheep named "Dolly," an exact genetic duplicate of the nucleus-donating ewe.

Study Guide #6
GENETIC ENGINEERING IN HUMANS

A pathogen is anything such as a virus or a bacterium that causes an immune system response. On the surface of pathogens and certain molecules that elicit an immune response are specific molecules called antigens, which trigger the response. Traditionally, vaccines were made from dead or weakened pathogens. This way, the antigens activate the body's immune system, causing it to produce antibodies to protect against later infections, but the dead or weakened pathogens do not cause disease. Unfortunately, accidents sometimes happen when the viruses in the vaccine are incompletely killed or revert to a virulent, or disease-causing form. With genetically engineered vaccines, only the antigens are produced, hence the risk of accidental infection is eliminated. Whole viruses lacking their destructive genes have also been designed. The viruses can then be administered in a vaccine without the risk of infection. Scientists are also attempting to insert genes for antigens from several different viruses into a single virus that can be used to vaccinate against several different viruses at the same time.



Another application of genetic engineering for humans is **gene therapy**. Gene therapy involves the use of genes to treat primarily genetic disorders at present, but may be used in the future to treat other types of disorders as well. Several techniques are being explored for transferring genetic material into human cells. One promising technique involves the use of **liposomes,** which are DNA coated with fatty substances called lipids. The liposomes allow the DNA to pass through the cell membrane where it would normally be repelled. However, the use of liposomes, as well as other techniques, is still experimental. Most gene therapy at present utilizes specially modified viruses for vectors.



Researchers have been able to utilize virus vectors with marginal success in the treatment of **cystic fibrosis.** Cystic fibrosis is a genetic disorder that causes excess mucus production, leading to accumulation and restriction of air flow in the lungs. Functional copies of the gene from people without the disease were isolated, cloned in bacteria, and eventually placed into a virus which is administered in the form of a nasal spray.

Quiz #1
DNA OVERVIEW

1. DNA always consists of a single long chain of molecules.

   A. True
   B. False

2. Adenine always bonds with which of the following nucleotides?

   A. cytosine
   B. guanine
   C. thymine
   D. all of the above

3. Nucleotides consist of a sugar, a phosphate group and a _____.

   A. nitrogenous base
   B. hydrogen group
   C. double helix
   D. gene

4. The DNA of all organisms is composed of the same four nucleotides; only the sequence of nucleotides differs from organism to organism.

   A. True
   B. False

5. The long strands of DNA typically exist in cells as condensed structures referred to as _____.

   A. chromosomes
   B. genes
   C. proteins
   D. RNA

6. Proteins can function as _____.

   A. enzymes
   B. cellular transporters
   C. structural elements
   D. all of the above

7. Nucleotide sequences of DNA that code for a particular trait or protein are called _____.

   A. chromosomes
   B. genes
   C. liposomes
   D. strands

8. During gene expression, the sequence of nucleotides is copied in a process known as _____.

   A. transportation
   B. impression
   C. transcription
   D. migration

9. Proteins are composed of molecules known as amino acids.

   A. True
   B. False

10. The intentional alteration or transfer of genetic material from one organism to another is called _____.

   A. phenotypic transposition
   B. genetic engineering
   C. Gene mapping
   D. gene expression

Quiz #2
GENETIC ENGINEERING OVERVIEW

1. Gene transfer is the oldest known form of genetic engineering.

   A. True
   B. False

2. Mutations are changes in the nucleotide sequence of DNA.

   A. True
   B. False

3. DNA coding for a gene from one organism that is being transferred to a new organism is called_____.

   A. selective DNA
   B. RNA
   C. altered DNA
   D. foreign DNA

4. DNA can be cut into fragments by powerful enzymes known as _____.

   A. polymerase
   B. restriction enzymes
   C. antibodies
   D. fragmentation enzymes

5. Single-stranded pieces of DNA left on both sides of a restriction fragment of DNA are called _____.

   A. sticky ends
   B. blunt ends
   C. waste fragments
   D. vectors

6. Clones can either be identical pieces of DNA or entire identical organisms.

   A. True
   B. False

7. Which of the following are used as cloning vectors?

   A. gene gun
   B. virus
   C. plasmid
   D. all of the above

8. Viruses are composed of DNA or RNA surrounded by a _____.

   A. lipid sheath
   B. protein coat
   C. gene layer
   D. cell wall

9. The enzyme that "glues" together the sugar-phosphate backbone of recombined DNA is _____.

   A. ligase
   B. restriction enzyme
   C. vector
   D. all of the above

10. DNA from two organisms which has been re-combined is called _____.

   A. sequential DNA
   B. RNA
   C. recombinant DNA
   D. none of the above

Quiz #3
GENETIC ENGINEERING IN MICROBES

1. A/An _____ is a substance that normally eliminates bacteria.

   A. enzyme
   B. antibiotic
   C. vector
   D. nucleosome

2. _____ are small, circular pieces of DNA.

   A. Chromosomes
   B. Plasmids
   C. Lipids
   D. Genes

3. The transfer of DNA into cells is called _____.

   A. transvection
   B. transmigration
   C. cloning
   D. transformation

4. Electroporation is the use of electrical current to create pores in cells.

   A. True
   B. False

5. The shotgun approach to gene cloning is so named because of the gene gun that scientists use for gene transfer.

   A. True
   B. False

6. Bacterial cells containing a plasmid with an antibiotic-resistant gene are less likely to grow in the presence of an antibiotic than bacteria without the plasmid.

   A. True
   B. False

7. A large collection of clones representing many or all of an organism's genes is called a _____.

   A. gene sequence
   B. gene library
   C. chromosome
   D. gene train

8. DNA and RNA probes often have radioactive molecules attached to them so that they can be easily identified.

   A. True
   B. False

9. Bacteria always express foreign DNA.

   A. True
   B. False

10. Molecules which seek out and identify a particular molecule or piece of DNA are referred to as _____.

    A. probes
    B. seekers
    C. clones
    D. communicators

Quiz #4
GENETIC ENGINEERING IN PLANTS

1. Plasmids are naturally found in plants.

   A. True
   B. False

2. Plant cells that have lost their cell wall are called _____.

   A. lipids
   B. leucoplasts
   C. microsomes
   D. protoplasts

3. _____ organisms are organisms which have been engineered with genes from another organism.

   A. Transgenic
   B. Foreign
   C. Cloned
   D. Genetic

4. The Ti plasmid naturally transfers part of its DNA to plant cells.

   A. True
   B. False

5. In order to use the Ti plasmid in gene transfer, scientists must first_____.

   A. convert the plasmid to a lipid
   B. remove the tumor-causing DNA
   C. submit the plasmid to electroporation
   D. attach radioactive probes

6. Nitrogen fixation is difficult to genetically engineer because _____.

   A. plants can't be genetically engineered
   B. the trait is controlled by plasmids
   C. many different genes are involved in the trait
   D. none of the above

7. Electroporation has no effect on plant cells.

   A. True
   B. False

8. *Bacillus thuringiensis* produces crystal-like proteins that_____.

   A. destroy plant tissues
   B. break down cell walls
   C. interfere with gene transfer
   D. kill certain insect larvae

9. Tomatoes have been genetically engineered to last longer by _____.

   A. removing the genes which cause spoilage
   B. cloning freshness genes from other plants
   C. creating a mirror image of the gene that causes spoilage, thus preventing its translation when the mirror image gene product binds to that of the spoilage gene
   D. adding antibiotic plasmids

Quiz #5
GENETIC ENGINEERING IN ANIMALS

1. Pigs have been engineered to produce organs coated in human proteins for use in transplants.

   A. True
   B. False

2. Dolly, the cloned sheep, was produced from a cell taken from _____.

   A. the udder of a mature sheep
   B. an egg
   C. an embryo
   D. none of the above

3. Before genetic engineering, vaccines were made from _____.

   A. parts of viruses
   B. dead viruses
   C. altered viruses
   D. all of the above

4. An antigen is _____.

   A. similar to a gene
   B. a protein or other substance which stimulates the immune system.
   C. a substance which causes mutations
   D. a substance used as a genetic probe

5. Animals, unlike plants and bacteria, contain plasmids.

   A. True
   B. False

6. Sheep have been engineered to produce milk containing specific human proteins used to treat genetic forms of _____ and _____.

   A. cancer and AIDS
   B. blindness and deafness
   C. hemophilia and emphysema
   D. mental retardation and leukemia

7. Mice and rats have been engineered to develop cancer and other human diseases to aid in research.

   A. True
   B. False

8. When bovine growth hormone is injected into dairy cows, milk production is not increased.

   A. True
   B. False

Quiz #6
GENETIC ENGINEERING IN HUMANS

1. Pituitary dwarfism in humans can be treated through administering _____.

   A. human growth hormone
   B. bovine growth hormone
   C. antibiotics
   D. insulin

2. People with certain forms of diabetes can be treated with injections of _____.

   A. human growth hormone
   B. plasmids
   C. insulin
   D. antibodies

3. The use of genes to treat disease is called _____.

   A. gene mapping
   B. genetic engineering
   C. gene therapy
   D. transformation

4. _____ are DNA coated in fatty acids.

   A. Viruses
   B. Chromosomes
   C. Genes
   D. Liposomes

5. Thus far, gene therapy has been most effective in treating genetic disorders caused by a single faulty gene.

   A. True
   B. False

6. Cystic fibrosis affects people with faulty genes for _____.

   A. hemoglobin production
   B. mucus production
   C. insulin production
   D. liposome production

7. A nasal spray containing a genetically engineered virus for treating cystic fibrosis has provided temporary relief from the disease symptoms.

   A. True
   B. False

8. Today, antigenic proteins from certain pathogens can be administered as vaccines instead of using weakened or dead viruses.

   A. True
   B. False