UNIT IV: FOUNDATIONS OF GENETIC ENGINEERING

Lesson 1: The Mechanics of Cells and DNA

Competency/Objective: Identify the parts of a cell, including DNA, and their functions.

Study Questions

1. **What are the parts of a cell and their functions?**

2. **What is DNA?**

3. **What is the structure and function of DNA?**

4. **How does DNA replicate?**

References

1. *Biotechnology: Applications in Agriculture (Student Reference)*. University of Missouri-Columbia: Instructional Materials Laboratory, 1998, Unit IV.

2. Transparency Masters

   a) TM 1.1: The Parts of a Cell
   b) TM 1.2: The Cellular Chain of Command
   c) TM 1.3: DNA Replication

3. Activity Sheet

   a) AS 1.1: Comparing Plant and Animal Cells

UNIT IV: FOUNDATIONS OF GENETIC ENGINEERING

Lesson 1: The Mechanics of Cells and DNA

TEACHING PROCEDURES

A. Introduction

Just as an automobile mechanic must understand the basics of how an engine works, so the biotechnology researcher must understand the cell, its parts, and how they work. This lesson will provide a review of concepts taught in biology and will lay the foundation for future lessons on the manipulation of genetic material.

B. Motivation

1. Use a model of DNA to illustrate its structure. A model can be purchased, or a flat model of DNA can be put together using a series of cards with the base pairs written on them. Students could also hold these cards in the appropriate position to simulate the double helix structure.

2. To observe cell walls in a plant cell, slice a carrot very thinly, so that light can easily be seen through it. Freeze the carrot slices. Take a frozen slice and place it under a microscope. Quickly observe the cell walls as they deteriorate when the cells thaw.

C. Assignment

D. Supervised Study

E. Discussion

1. Ask students to name as many parts of a cell as they can. Have a student write these on the board. Add any parts that they might have missed. Use TM 1.1 to illustrate the parts. Have students complete AS 1.1.

What are the parts of a cell and their functions?

a) Animal cell
   1) Cell (plasma) membrane - controls movement of materials into and out of the cell
   2) Cytoplasm - the contents of the cell, excluding the nucleus; contains fluid that helps control the movement of substances within the cell and includes the organelles
   3) Mitochondria - powerhouses of the cell; break down nutrients to provide energy
   4) Endoplasmic reticulum - network of membranes that transports materials within the cell; location of the ribosomes
   5) Ribosomes - primary location for protein synthesis
   6) Golgi apparatus - packages protein molecules for movement within and outside the cell
   7) Vacuole - stores water, enzymes, pigments, and other substances
   8) Nucleus - control center for the cell; contains chromosomes
   9) Chromosomes - tightly wrapped pieces of DNA; location of genes
   10) Genes - a segment of DNA on a chromosome that produces a polypeptide (protein) and is responsible for the expression of genetic traits

b) Plant cell - contains all of the structures found in animal cells with two additions
   1) Chloroplasts - contain chlorophyll, which is used in photosynthesis
   2) Cell wall - rigid outer layer that provides support for the plant cell and works collectively with other cells to support the plant

c) Bacteria cell
    1) Cell wall
    2) Cell membrane
    3) Ribosomes
    4) Nucleoid region - chromosomal material not contained within a nucleus
    5) Plasmid - small circular piece of DNA that codes for specific traits and replicates independently of chromosomal DNA; normally contains only a few genes

2. Ask a student to look up the definition of DNA, or deoxyribonucleic acid, in a dictionary or a CD-ROM encyclopedia.

What is DNA?

a) DNA (deoxyribonucleic acid) is the genetic material of the cell composed of small chemical units called nucleotides, which consist of three parts.
    1) A phosphate group
    2) A sugar (called deoxyribose) unit
    3) One of four nitrogen base units (adenine, guanine, thymine, and cytosine)
b) A single strand of DNA may contain more than 100 million base pairs.

3. Ask students to list as many words on the board as they can that describe what a DNA molecule looks like. Use TM 1.2 to illustrate the structure of DNA.

What is the structure and function of DNA?

a) Structure
    1) Two strands of nucleic acid are intertwined in a double helix structure that looks like a twisted or spiraling ladder.
    2) The phosphate and sugar units form the sides of the ladder, while the nitrogen base units form the rungs.
    3) The base units are found in one of two bonding arrangements, adenine bonded to thymine or guanine bonded to cytosine; hydrogen bonds join the base units.
b) Function
    1) DNA determines what types of proteins to build in protein synthesis.
        (a) DNA functions in sections called codons, which are sets of three nucleotides that code for one of the twenty amino acids.
        (b) These amino acids are then linked together to form polypeptides.
        (c) Two or more polypeptides are linked together to form a protein.
    2) In DNA replication, DNA makes a copy of itself to pass on its code to new cells formed by cell division.

4. Using TM 1.3, ask students why DNA needs to replicate. Explain that DNA must replicate, or each time a cell divided, it would lose half its DNA. Next, have students explain how DNA replication takes place.

How does DNA replicate?

a) A protein made by the cell binds to a place on the DNA called the origin.
b) An enzyme begins to break the hydrogen bonds that hold the two strands of the helix together, causing the double helix to “unzip.”
c) A complex enzyme (DNA polymerase) binds to each DNA strand segment and begins to add a new base unit to the strand; the added base must be compatible with the base on the parent DNA strand.
d) Another enzyme then bonds the new nucleotides with the parent DNA strand.
e) Each DNA molecule now consists of one parent strand and one newly formed strand.

F. Other Activities

1. Have students use cardboard puzzle pieces made to look like bases to show how DNA replicates.

2. Have students research and present a report about the discovery of the structure of DNA by Watson and Crick.

G. Conclusion

Plant, animal, and bacteria cells are all different in some respects. However, they all contain at least one chromosome, and therefore they contain DNA. The DNA in a plant, animal, or bacteria cell is essentially the same except for the base pair sequence it contains. This similarity of DNA makes the manipulation and transfer of DNA between different life-forms possible. If the structure or function of plant DNA was different from animal DNA, then DNA from a plant could not be spliced into the DNA of an animal. A clear understanding of the makeup of cells and a working knowledge of the structure and function of DNA is necessary to understand genetic manipulation.

H. Answers to the Activity Sheet

Student pictures will vary, but they should show that the students observed some parts of the cells.

I. Answers to the Evaluation

1. j
2. k
3. g
4. e
5. c
6. f
7. l
8. a
9. b
10. h
11. i
12. d
13. a
14. d
15. b

16. The steps in the replication of DNA are as follows:

- A protein binds to a place on the DNA called the origin.
- An enzyme begins to break the hydrogen bonds that hold the two strands of DNA together, unzipping the double helix.
- As the DNA strands are being unzipped, a complex enzyme (DNA polymerase) binds to each DNA strand segment and begins to add a new base unit to the strand. The added base must be compatible with the base on the parent DNA strand.
- Another enzyme then bonds the new nucleotides with the parent DNA strand.

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EVALUATION

Match the correct function on the right to the cell part on the left by writing the letters in the blanks.

1. _____ Cell wall
2. _____ Cell membrane
3. _____ Cytoplasm
4. _____ Mitochondria
5. _____ Endoplasmic reticulum
6. _____ Ribosomes
7. _____ Golgi apparatus
8. _____ Vacuole
9. _____ Plasmid
10. _____ Nucleus
11. _____ Chromosome
12. _____ Gene

a. Stores water, enzymes, pigments and other substances
b. Small circular piece of DNA that contains a few genes
c. A membrane that transports molecules
d. Segment of DNA that is responsible for the expression of a trait
e. Powerhouse of the cell
f. Primary location for protein synthesis
g. Contents of the cell, excluding the nucleus
h. Control center for the cell
i. Location of genes
j. Provides support for plant cells
k. Controls the movement of materials into and out of the cell
l. Packages protein molecules for movement within and outside the cell

Circle the letter that corresponds to the best answer.

13. The unit of a DNA molecule that contains the code for building proteins is the:

    a. Nitrogen base unit.
    b. Phosphate unit.
    c. Sugar unit.
    d. Potassium unit.

14. When examining the structure of DNA, which of the following bonding arrangements is found in the DNA molecule?

    a. Guanine bonded to thymine
    b. Cytosine bonded to thymine
    c. Adenine bonded to cytosine
    d. Guanine bonded to cytosine

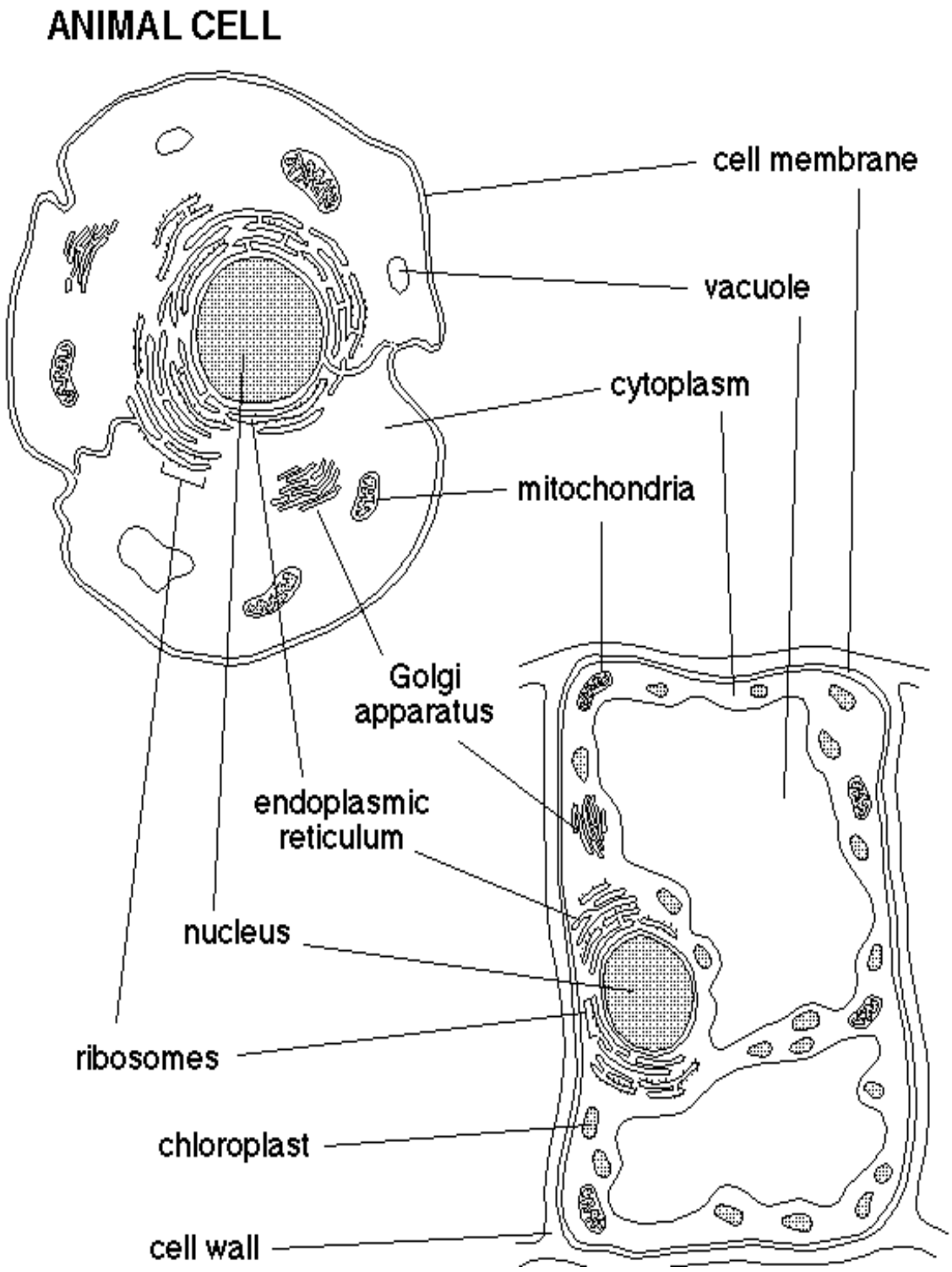
15. A codon is a:

a. Segment of DNA that is responsible for the expression of a trait.
b. Segment of DNA that is three base pairs long and codes for a specific amino acid.
c. Segment of DNA that codes for one of twenty polypeptides.
d. Segment of DNA that contains no base units.

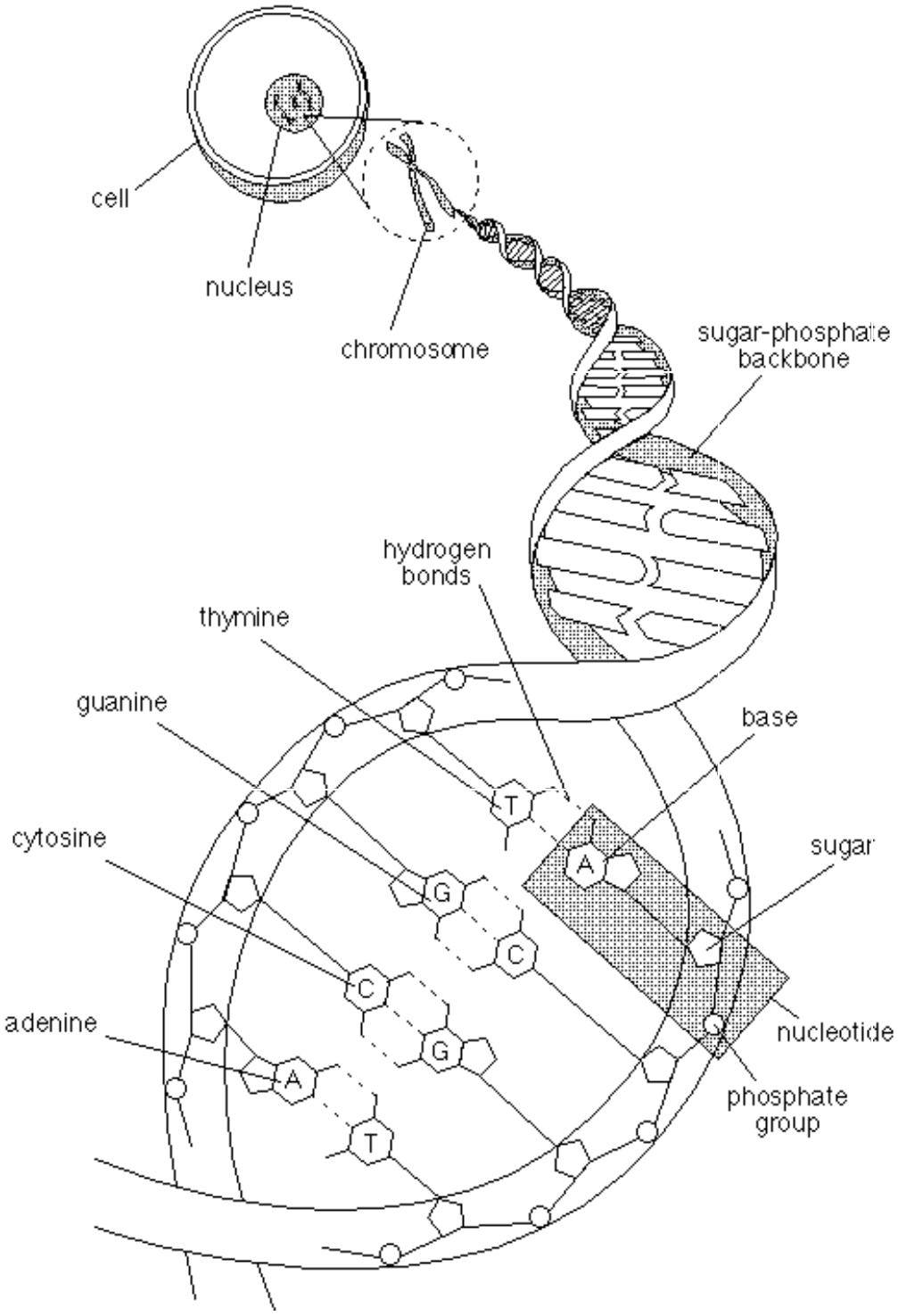
Complete the following short answer question.

16. What are the steps in DNA replication?

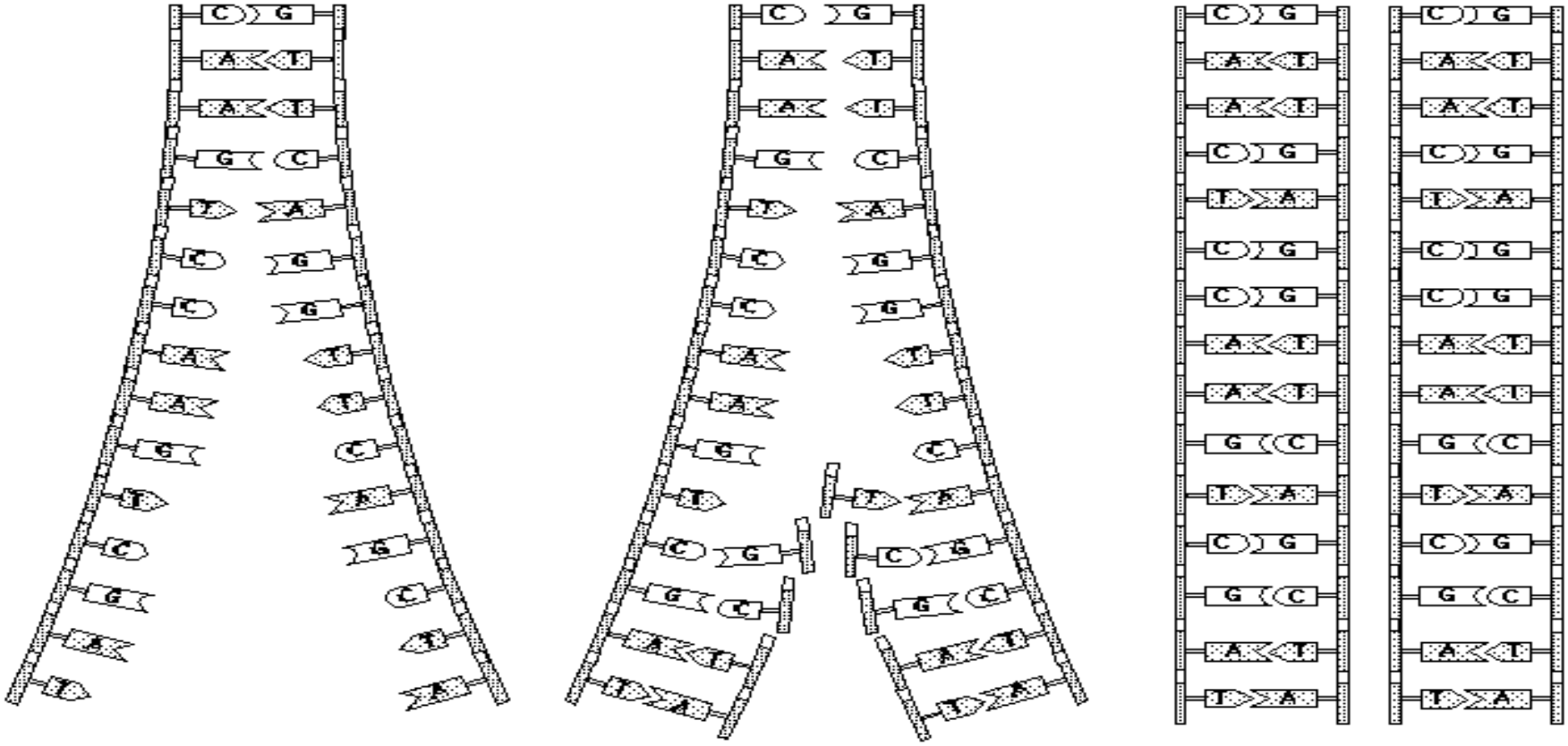
The Parts of a Cell



The Cellular Chain of Command



DNA Replication



Comparing Plant and Animal Cells

Objective: Identify the parts of plant and animal cells.

Materials and Equipment:

Microscope
2 glass slides
2 cover slips
Onion
Toothpicks
0.9 percent NaCl
Methylene blue

Procedure:

A. Red or Purple Onion Cells

1. Remove a paper thin section of an onion peel. Cut out a small piece about the size of a penny. Place this tissue sample on a clean glass slide. Add a drop of water and carefully place a cover slip over the specimen. **Gently** press out any air bubbles.

2. Examine the tissue using the low-power objective and then the high-power objective. Look for the nucleus, nuclear membrane, cytoplasm, and cell wall.

3. Draw a picture of one of the onion cells and label the cell parts that you observed.

B. Human Epithelial Cells

1. Carefully scrape the inside of your mouth with the flattened end of a toothpick. Place the scrapings in a drop of 0.9 percent NaCl on a clean glass slide. Repeat the scraping procedure four times and then place a cover slip over the specimens.

2. Add a drop of methylene blue to one edge of the cover slip. To pull the stain across the specimen, use a paper towel to begin absorbing liquid at the edge of the cover slip opposite the stain. Continue until the excess stain has been removed.

3. Examine the cells using the low- and high-power objectives. Draw and label the parts of the cells that you observe.

UNIT IV - FOUNDATIONS OF GENETIC ENGINEERING

Lesson 2: Cell Reproduction and Genetics

Competency/Objective: Explain how cells reproduce.

Study Questions

1. **What is mitosis?**
2. **What is meiosis?**
3. **How do mitosis and meiosis differ?**
4. **What are dominant and recessive genes?**
5. **What are homozygous and heterozygous gene pairs?**
6. **What are genotypes and phenotypes?**
7. **What are mutations?**

References

1. *Biotechnology: Applications in Agriculture (Student Reference)*. University of Missouri-Columbia: Instructional Materials Laboratory, 1998, Unit IV.
2. Transparency Masters
   a) TM 2.1: Mitosis
   b) TM 2.2: Meiosis
3. Activity Sheet
   a) AS 2.1: Mitosis and Meiosis

UNIT IV - FOUNDATIONS OF GENETIC ENGINEERING

Lesson 2: Cell Reproduction and Genetics

TEACHING PROCEDURES

A. Review

In the last lesson, the cell and its components were examined in some detail. This lesson focuses on the two ways in which cells reproduce and how this reproduction can pass on genetic information and the traits seen in the organism's offspring.

B. Motivation

When discussing cell reproduction, the obvious question to ask is why body cells need to reproduce at all. If a body cell can grow and get bigger, why does it need to reproduce itself? Think for a moment about a large car factory. Why do manufacturers like General Motors or Ford have different factories in the Midwest? Why not one big factory?

Several reasons exist for having many factories or many cells. First, the managers of the factory (the nucleus of the cell) can only control a limited workload. The operations of the factory (the making of proteins in the cell) cannot increase above the capacity that the management (cell nucleus) can handle. Another reason for having more than one is so that the supply and export of products is not slowed. The larger a factory gets the more raw materials it demands (just as the larger a cell gets the more nutrients, oxygen, and other raw materials it demands). At the same time, the export of products also increases, and these two factors slow the movement of raw materials and products into and out of the factory (cell).

C. Assignment

D. Supervised Study

E. Discussion

1. Ask students if they know how hair, fingernails, skin, or muscles grow. Use TM 2.1 to show the stages of mitosis and how tissues grow.

What is mitosis?

a) Mitosis is a type of cell division in which somatic cells divide and form two new cells; each new cell contains the same number of paired chromosomes as the parent cell, or two complete sets of chromosomes (a diploid number of chromosomes).
b) Stages of mitosis (Hint: Remember PMAT.)
    1) Prophase
        (a) Chromosomes coil and condense to form a double-stranded structure consisting of two paired chromatids connected at a body called a centromere.
        (b) The nuclear membrane dissolves gradually.
        (c) A network of microtubules begins forming around centrioles, which start to move to opposite ends of the cell; the network of microtubules is called a spindle.
        (d) Spindle fibers extend out from the centrioles toward the center of the cell.
    2) Metaphase
        (a) Chromosomal units move to the center of the cell and form a line between the two poles.

(b) Each spindle fiber attaches to a centromere.

3) Anaphase
   (a) The centromeres break, allowing the spindle fibers to pull apart the chromatids.
   (b) The chromosomes move toward opposite poles of the cell.
   (c) The poles move farther apart, elongating the cell.
   (d) At the end of anaphase, the two poles each have a complete set of chromosomes.
4) Telophase
   (a) In animal cells, the cell membrane pinches together, dividing the cell into two cells.
   (b) In plant cells, a cell plate forms in the middle of the cell and begins to divide it into two cells; a cell membrane forms on both sides of the cell plate, and the cell plate changes to a cell wall.
   (c) Nuclear membranes develop in the daughter cells.
   (d) The chromosomes uncoil and lose their distinct outlines.

2. Ask students how sex cells divide. Use TM 2.2 to show the stages of meiosis. Have students complete AS 2.1. When doing the activity, make sure to observe each phase before allowing students to move on to the next one. The instructor may want to make up a poster showing the different phases so that students can use it as a model.

What is meiosis?

a) Meiosis is a type of cell division that produces gametes (sex cells). It has two phases of cell division, producing four gametes. The gametes contain half the number of chromosomes found in the parent cell, or a single set of chromosomes, and are referred to as haploid cells.

b) Meiosis I
   1) Prophase I
      (a) Homologous chromosomes pair together and form a tetrad, a grouping of four chromatids side by side.
      (b) Nonpaired chromatids may exchange segments in a process called "crossing over."
      (c) The centrioles move apart and the spindle forms.
      (d) The nuclear membrane dissolves.
   2) Metaphase I
      (a) Homologous chromosomes line up in the center of the cell.
      (b) The spindle fiber ends attach to centromeres.
   3) Anaphase I - Homologous chromosomes separate and are pulled to different poles.
   4) Telophase I
      (a) The cell plate and/or cell membrane forms, creating two haploid daughter cells.
      (b) Nuclear membranes form.

c) Meiosis II
   1) Prophase II
      (a) The spindle fibers develop.
      (b) Paired chromatids move to the center of the cell.
   2) Metaphase II
      (a) The chromosomal units line up in a row between the two poles.
      (b) They become attached to the spindle fibers.
   3) Anaphase II - Chromatids separate and move toward opposite poles.
   4) Telophase II
      (a) The center of each cell closes off with the formation of a cell membrane or cell plate.
      (b) Nuclei form.

(c) Chromosomes uncoil.

3. Have students compare mitosis and meiosis and list the differences.
How do mitosis and meiosis differ?

a) Mitosis produces two cells from one parent cell, while meiosis produces four cells from one parent cell.
b) Mitosis produces diploid somatic cells, while meiosis produces haploid gametes.
c) Mitosis produces two identical cells, and meiosis produces four nonidentical cells.
    1) During mitosis, chromosomes contribute an identical chromosome to each daughter cell.
    2) In meiosis, homologous chromosomes split and contribute nonidentical chromosomes to each daughter cell.
d) Meiosis allows genes to cross over, while mitosis does not.

4. Ask students to define the word dominant. Relate this definition to dominant and recessive genes.

What are dominant and recessive genes?

a) Chromosomes work in pairs.
b) Each chromosome has a homologous chromosome that has genes that code for the same information but somewhat differently.
c) Each gene in a gene pair is either dominant or recessive.
d) Dominant genes are expressed; they mask the expression of a recessive gene.
e) Recessive genes are genes that are not expressed, or apparent in the traits of the animal.

5. Ask students to define the terms homozygous and heterozygous.

What are homozygous and heterozygous gene pairs?

a) Either of the two possible expressions of a gene or multiple genes that code for a specific trait is called an allele.
b) An organism with two dominant alleles or two recessive alleles is homozygous for that specific trait; they are either homozygous dominant or homozygous recessive.
c) An organism with one dominant allele and one recessive allele is heterozygous for that trait.

6. Have students explain and give examples of a genotype and a phenotype.

What are genotypes and phenotypes?

a) Genotype - The specific combination of alleles for a trait.
b) Phenotype - The appearance or expression of a trait as determined by the genotype.

7. Ask student if they believe that all mutations are bad. Point out that mutations such as seedless grapes and oranges are not harmful.

What are mutations?

a) Mutations are alterations in the nucleotide sequence found in a DNA molecule; they may be from the insertion, deletion, or miscoding of a base unit during replication.
b) Mutations can occur during replication in mitosis or meiosis.
    1) Mutations during mitosis are not inheritable; they affect the daughter cell and any cells descending from it.

2) Mutations prior to meiosis are passed on to an organism's offspring if the mutant gamete is fertilized.

F. Other Activities

Review the use of Punnet squares to further discuss genotype and phenotype.

G. Conclusion

An understanding of cell reproduction and the basic genetic concepts discussed in this lesson suggests how genetic manipulation must be done. To add a gene to an entire plant or animal, genetic manipulation must be done before or during meiosis. Gene interaction is quite complex, and genetic manipulation is even more complex.

H. Answers to Activity Sheet

AS 2.1

1. a. Mitosis produces two cells from one parent cell, while meiosis produces four cells from one parent cell.
   b. Mitosis produces diploid somatic cells, while meiosis produces haploid gametes.
   c. Mitosis produces two identical cells, and meiosis produces four nonidentical cells.
   d. Meiosis allows genes to cross over, while mitosis does not.

2. In meiosis I, homologous chromosomes are separated, and a double-stranded chromosomal unit is passed on to the new cells. In meiosis II, the chromatids split apart, with each new cell receiving one chromosome.

I. Answers to the Evaluation

1. a
2. d
3. b
4. a
5. c

6. Mutations are alterations in the nucleotide sequence found in a DNA molecule; they may be from the insertion, deletion, or miscoding of a base unit during replication.

7. a) Prophase
   b) Metaphase
   c) Anaphase
   d) Telophase

8. Students may list any two of the following:

   - Mitosis produces two cells from one parent cell, while meiosis produces four cells from one parent cell.
   - Mitosis produces diploid somatic cells, while meiosis produces haploid gametes.
   - Mitosis produces two identical cells, and meiosis produces four nonidentical cells.
   - Meiosis allows genes to cross over, while mitosis does not.

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EVALUATION

Circle the letter that corresponds with the best answer.

1. Meiosis can be defined as a type of cell division in which:

    a. Gametes are produced.
    b. Somatic cells are produced.
    c. Two identical cells are produced.
    d. Diploid cells are produced.

2. One difference between animal and plant cells during the last phase of mitosis is that in animal cells the cell membrane pinches in to divide the cell, while in plant cells:

    a. Cell membranes do not exist.
    b. The cell membrane grows out of the center of the cell, dividing it.
    c. The nuclear wall grows to divide the cell.
    d. The cell plate grows to divide the cell.

3. What is the term used to describe when chromosomes exchange segments during meiosis?

    a. Haploid exchange
    b. Crossing over
    c. Centromere replication
    d. Mutation

4. If a plant has a recessive allele for a dwarf plant and a dominant allele for a tall plant, how tall would the plant be?

    a. Tall
    b. Short
    c. Medium height
    d. It is impossible to tell how tall it will be.

5. What is the appearance or expression of a trait in an organism called?

    a. Chromotype
    b. Genotype
    c. Phenotype
    d. Heterozygous

Complete the following short answer questions.

6. What are mutations?

7. What are the four stages of mitosis, in the order in which they occur?

   a.

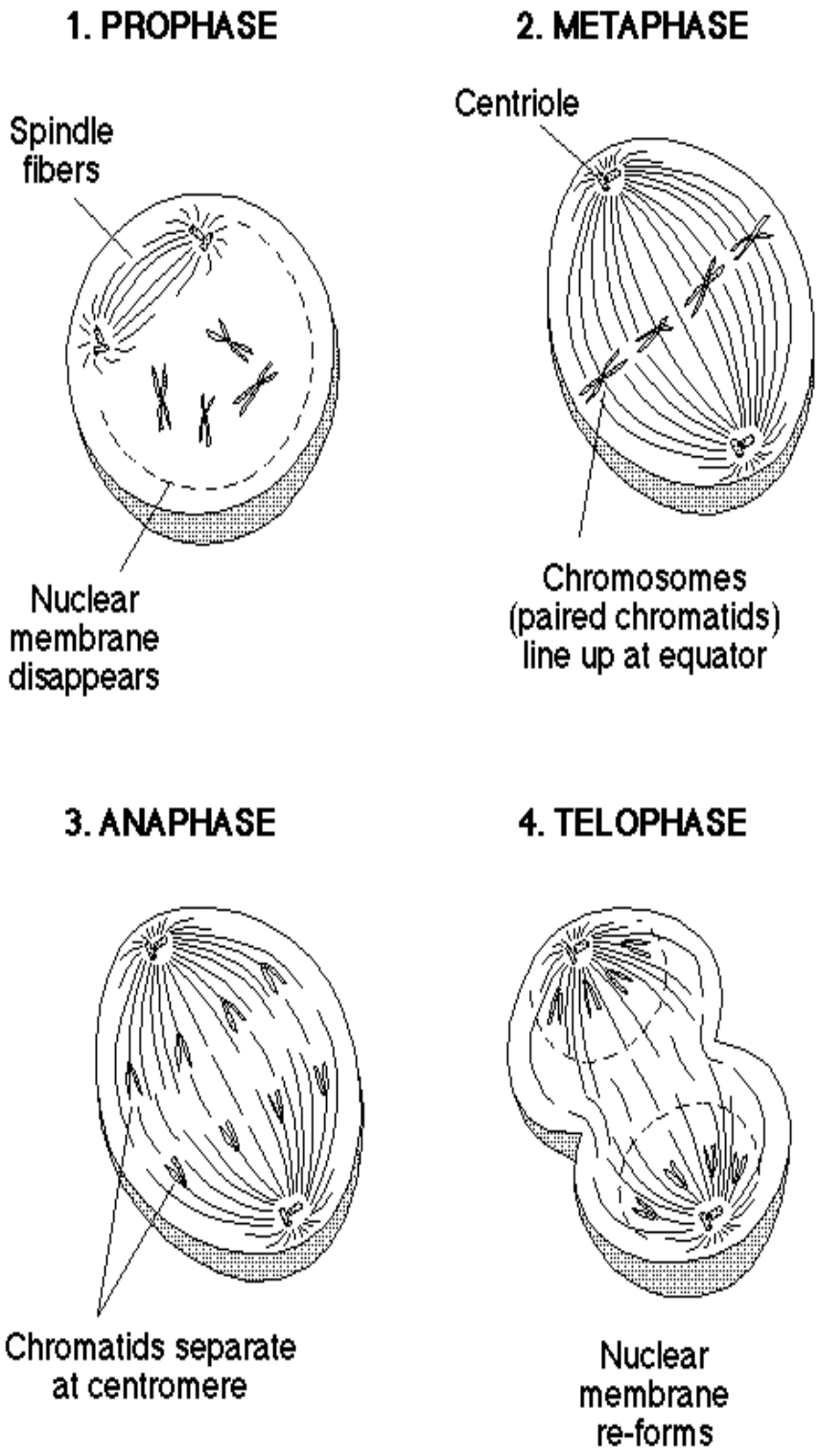
   b.

   c.

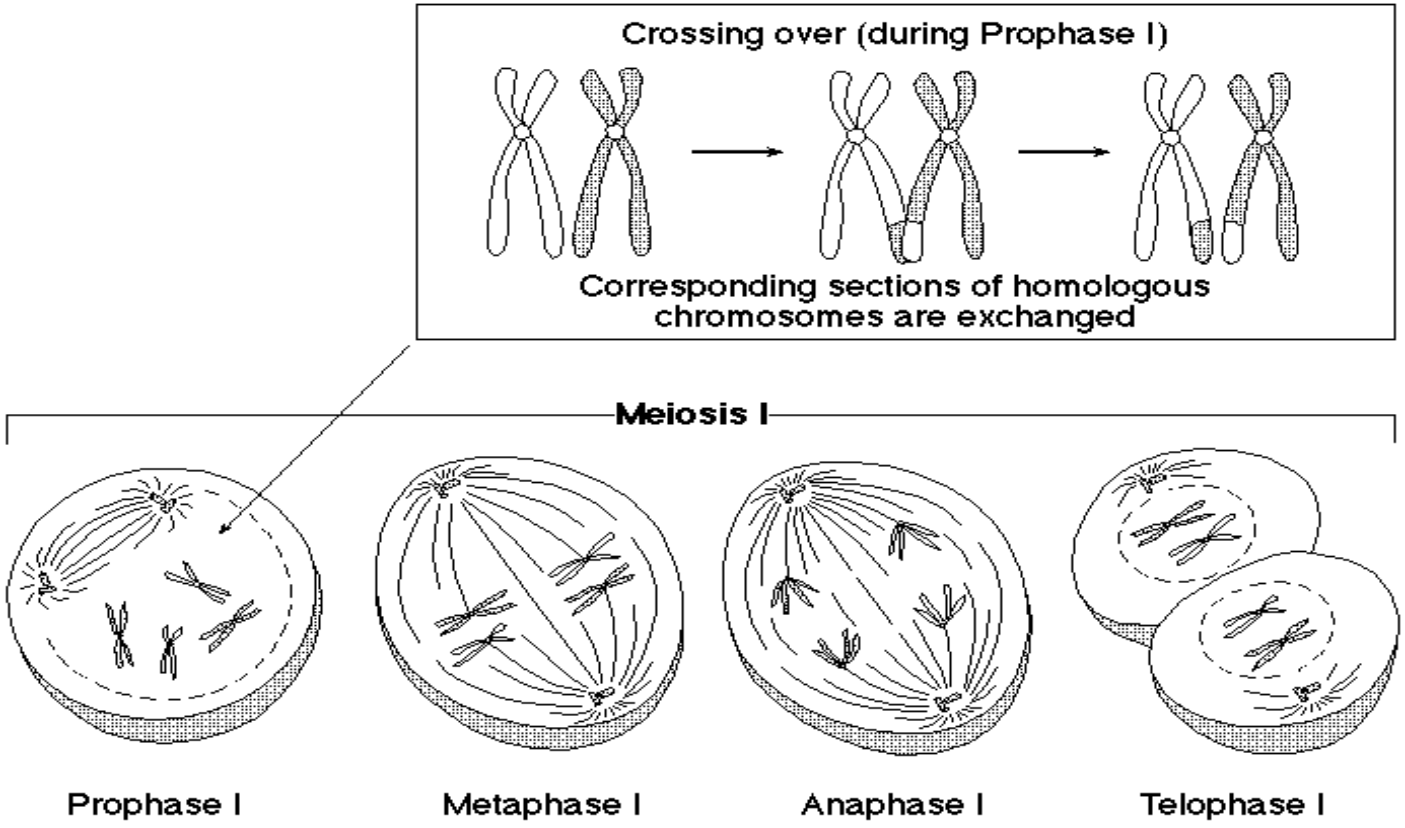
   d.

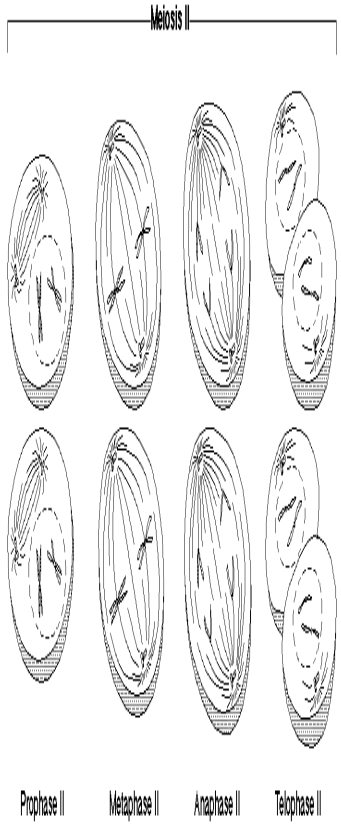
8. What are two ways in which mitosis and meiosis differ?

Mitosis


1. PROPHASE
Spindle fibers
Nuclear membrane disappears
2. METAPHASE
Centriole
Chromosomes (paired chromatids) line up at equator
3. ANAPHASE
Chromatids separate at centromere
4. TELOPHASE
Nuclear membrane re-forms

Meiosis


Crossing over (during Prophase I)
Corresponding sections of homologous chromosomes are exchanged
Meiosis I
Prophase I
Metaphase I
Anaphase I
Telophase I


Meiosis II
Prophase II
Metaphase II
Anaphase II
Telophase II

Lesson 2: Cell Reproduction and Genetics Name _____

Mitosis and Meiosis

Objective: Simulate the stages of mitosis and meiosis.

Materials and Equipment:

Pipe cleaners
Beads
Pieces of yarn

Procedure:

1. Using pipe cleaners, beads, and pieces of yarn, illustrate the four stages of mitosis. The pipe cleaners and beads should represent the chromosomes. The pieces of yarn can illustrate the nucleus, centrioles, and spindle fibers. Be ready to explain what happens in each of these four stages.

2. Use the pipe cleaners, beads, and yarn to illustrate the four stages of meiosis I. Be sure to show the differences that distinguish meiosis I from mitosis. Be ready to explain each stage of meiosis I.

3. Use the pipe cleaners, beads, and yarn to illustrate the four stages of meiosis II. Be sure to illustrate the crossing over of genes. Be ready to explain each stage of meiosis II.

Key Questions:

1. What are four differences that exist between mitosis and meiosis?

   a.

   b.

   c.

   d.

2. How does meiosis I differ from meiosis II in terms of the genetic material passed on to the new cells?

UNIT IV - FOUNDATIONS OF GENETIC ENGINEERING

Lesson 3: Genetic Modification

Objective/Competence: Describe the processes of genetic modification.

Study Questions

1. **What are gene mapping and gene sequencing?**
2. **How is DNA extracted?**
3. **What is restriction digestion?**
4. **What is gel electrophoresis?**
5. **What is gene splicing, and how is it accomplished?**

References

1. *Biotechnology: Applications in Agriculture (Student Reference)*. University of Missouri-Columbia: Instructional Materials Laboratory, 1998, Unit IV.
2. Transparency Master
   a) TM 3.1: Gel Electrophoresis
3. Activity Sheet
   a) AS 3.1: DNA Extraction

UNIT IV - FOUNDATIONS OF GENETIC ENGINEERING

Lesson 3: Genetic Modification

TEACHING PROCEDURES

A. Review

Lesson 2 discussed how cells reproduce and pass on genetic information. A spontaneous change in the genetic code of a cell or organism is called a mutation. Recombinant DNA technology provides researchers with the tools necessary to intentionally alter specific parts of the genetic code of a cell. This lesson will describe some of these tools.

B. Motivation

Gel electrophoresis can be dramatized by the use of an obstacle course. Configure student chairs or desks to form three obstacle courses. Divide the students into three groups, with two to four students in the first group, four to eight in the second group, and eight to twelve in the third group. All groups should start the obstacle course at the same time and should be instructed not to touch any chair or desk. When the winning team reaches the finish line, all teams must freeze. Explain that the obstacle course represents the agarose gel through which the DNA fragments must pass in gel electrophoresis. The longer the fragment, the slower it will move across the gel.

C. Assignment

D. Supervised Study

E. Discussions

1. Ask students to describe gene mapping and gene sequencing.

What are gene mapping and gene sequencing?

a) Gene mapping is the process of finding the location of genes for specific traits on the chromosomes of an organism. Researchers use gene mapping to select specific genes for modification.
b) Gene sequencing is a process that shows the order of all the base units (A, T, C, G) as they line up on a particular gene. It allows scientists to recognize how to cut out a particular gene or gene fragment.

2. Ask students if DNA is a chemical substance. Because it is a chemical substance, it can be extracted chemically. Use AS 3.1 to allow students to perform a DNA extraction.

How is DNA extracted?

a) The cell membrane or cell wall must be broken down to release the cytoplasm, and the nuclear membrane must also be broken to release the chromosomes.
    1) This can be accomplished with a surfactant, which is a fatty acid compound like detergent.
    2) Heat accelerates this process.
b) Next, a protease must be used to split the protein contents of the cell, including protein molecules called Histones, around which the DNA strands are wrapped.
c) The last step involves separating the DNA from the other cell components. Cold alcohol is added to the cellular solution; the DNA clumps together and rises to the top of the alcohol.

3. Ask students what scissors are used for. Discuss how restriction digestion works.

What is restriction digestion?

a) Restriction digestion is the process of cutting DNA into smaller fragments with restriction enzymes.
b) Restriction enzymes are essentially biochemical scissors; each restriction enzyme cuts DNA at a different sequence of base pairs.
c) Researchers use restriction enzymes to cut genes or DNA fragments out of extracted DNA strands.

4. Ask students to recall the lesson in which DNA fingerprinting was discussed. Explain that the bands that make up the DNA fingerprint are a result of gel electrophoresis. Discuss the process of gel electrophoresis. Use TM 3.1 to illustrate how gel electrophoresis works.

What is gel electrophoresis?

a) Gel electrophoresis is a process in which an electric current is applied to a gel to separate different lengths of DNA fragments into groups; researchers can then recover a desired gene or gene fragment.
b) It requires an electrophoresis box, a buffer solution, a special power supply, and a gel made from agarose or another agent.
c) One end of the electrophoresis box has a positive pole and the other a negative pole.
d) DNA fragments to which a dye has been added are placed in small wells or pockets at the end of gel nearest the negative pole, and an electric current is applied to the gel.
e) Since DNA fragments are negatively charged, they will be repelled away from the negative pole and attracted to the positive pole.
f) Short lengths of DNA will move through the gel faster than long lengths.
g) When the electric current is removed, the fragments of DNA of the same size will be grouped at a one spot on the gel, which is called a band.

5. Ask students to define the term splicing. Explain that gene splicing is like splicing two pieces of rope together using a third piece of rope.

What is gene-splicing, and how is it accomplished?

a) Gene-splicing is the process of inserting a piece of DNA into a chromosome of a cell.
b) Gene-splicing is also called ligation because the enzyme ligase is the biochemical glue that joins the pieces of DNA.
c) Gene-splicing requires several steps.
    1) The researcher cuts out a piece of DNA with a restriction enzyme; the correct enzyme must be used so that the DNA contains complementary bases.
    2) Gel electrophoresis must be performed to separate the DNA fragments by size and isolate the appropriate fragment.
    3) The researcher joins the ends of the selected fragment to the DNA being transformed through a chemical reaction called a ligase reaction.
    4) The result is a cell containing DNA from two different sources, which is therefore called recombinant DNA.

F. Other Activities

1. Use pop beads to show how restriction digestion works.

2. If possible, use restriction analysis and gel electrophoresis experiments such as those found in the curriculum guide *An Introduction to Biotechnology: A Unit for High School Students (Book Three)* published by Kendall/Hunt.

G. Conclusion

This lesson describes some of the most common techniques used by molecular biologists involved in biotechnology research. Knowledge of these techniques will provide a basic understanding of exactly how DNA is manipulated.

H. Answers to Activity Sheet

AS 4.1

1. The soap helped break down the cell membranes and release the chromosomes.

2. Heating the solution accelerated the breakdown of the membranes. Most of the lipids and proteins precipitated out of the solution. However, the solution needed to be cooled after heating to avoid the soap breaking down the DNA.

3. The meat tenderizer contains the enzyme papain, which breaks down proteins.

4. DNA is the only component of the cell that is not soluble in alcohol and therefore precipitates out.

5. (Students should speculate on this question but may not be able to come up with this answer.) The DNA was not pure because some of the other cellular components could have clung to the DNA. It must be washed in alcohol several times to be pure.

I. Answers to the Evaluation

1. d
2. b
3. a
4. c
5. d

6. a) The cell membrane or cell wall and nuclear membranes are broken down with the use of a surfactant.
   b) Protease is used to break down the protein contents of the cell, including histones, around which the DNA is wrapped.
   c) DNA is separated from the other cell components by adding cold alcohol to the cellular solution, which causes the DNA to clump together and rise to the top of the alcohol.

7. Gene sequencing is a process that shows the order of all the base units (A, T, C, G) as they line up on a particular gene.

8. Restriction digestion is the process of cutting DNA into smaller fragments with restriction enzymes.

EVALUATION

Circle the letter that corresponds to the best answer.

1. Which of the following is considered to be a type of biochemical scissors?

   a. Agarose gel
   b. Histones
   c. Ligase
   d. Restriction enzymes

2. When a researcher does gel electrophoresis, what causes the DNA fragments to move across the gel?

   a. Buffer solution flows across the gel, moving the DNA fragments.
   b. Negatively charged DNA is repelled away from the negative pole and attracted toward the positive pole of the electrophoresis box.
   c. Positively charged DNA is repelled away from the positive pole and attracted toward the negative pole of the electrophoresis box.
   d. A chemical reaction involving ligase causes the movement of the DNA.

3. Ligase is an enzyme that:

   a. Chemically joins two DNA fragments.
   b. Acts as a restriction enzyme.
   c. Is used in the gel electrophoresis buffer solution.
   d. Is used in DNA extractions to break down the lipid components of the cell.

4. Surfactants, which are similar to detergents:

   a. Break down the protein contents of the cell.
   b. Cause DNA to float to the top of a cellular solution.
   c. Break down the cellular membranes.
   d. Speed up the process of DNA extraction.

5. Gene mapping can be defined as the:

   a. Process that allows researchers to separate different lengths of DNA fragments into groups.
   b. Process of inserting a piece of DNA into a chromosome of a cell.
   c. Process that finds the order of all the base units as they line up on a particular gene.
   d. Process of finding the location of genes on the chromosomes of an organism.

Complete the following short answer questions.

6. What are the three major steps of the DNA extraction process?

   a.

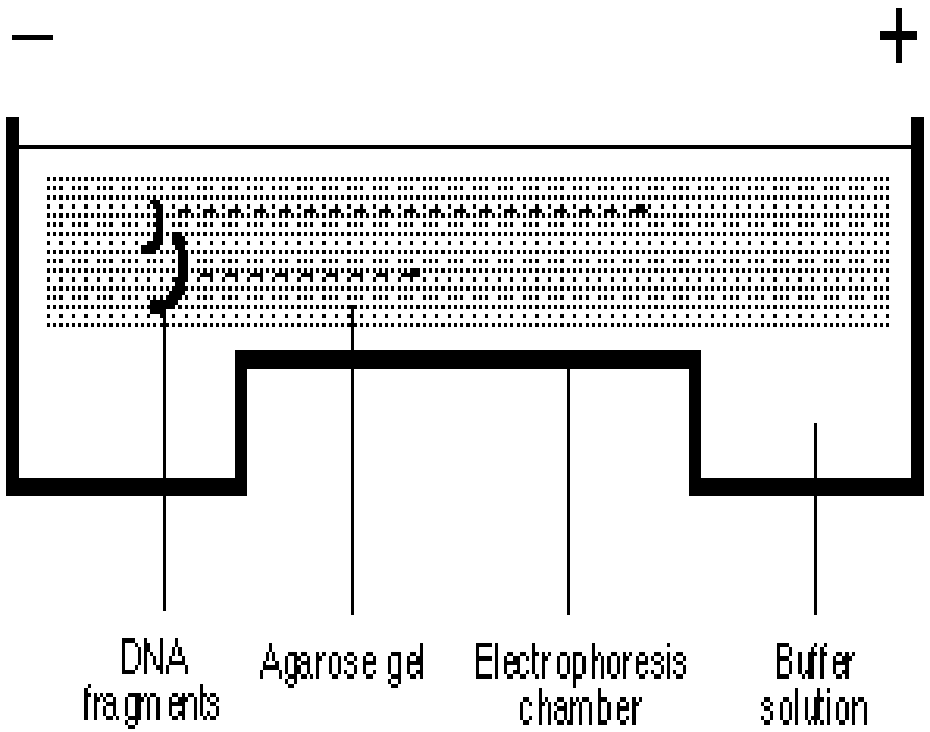
   b.

   c.

7. What is gene sequencing?

8. What is restriction digestion?

Gel Electrophoresis



Lesson 3: Genetic Modification Name _____

DNA Extraction

Objective: Perform DNA extraction.

Materials and Equipment:

1 hot water bath or hot plate set at 55 to 60 degrees Celsius
1 candy or laboratory thermometer (to regulate the hot water bath)
1 cold water bath (ice water in a large bowl)

Materials needed for each group of students:

1 glass or plastic graduated cylinder (100 ml)
1 plastic teaspoon
1 Pyrex glass or heat-resistant plastic beaker or flask (500 ml or larger)
1 piece of glass tubing bent to a 90 degree angle at the end
1 funnel or strainer with a coffee filter
2 flat toothpicks
1 medicine dropper or pipette
1 container of liquid soap (Woolite, laundry soap, or dish soap)
1 test tube for each student
½ medium onion, chopped (should not be finely chopped)
Refrigerated alcohol
Meat tenderizer

Procedure:

1. Observe the instructor as he or she performs steps 2-6.

2. Measure out 5 ml of soap and leave it in the graduated cylinder. Add ¼ teaspoon of salt to the soap. Next, add water to the soap until a total volume of 50 ml is reached. Set the cylinder aside. Place the onion in the beaker or flask. Pour the soap and salt water solution over the chopped onion.

3. Put the beaker or flask containing the onion solution into the hot water bath or onto the hot plate. Check to make sure that the temperature is approximately 55 to 60 degrees Celsius. The onion solution should be heated for 10 to 12 minutes. If the solution is heated for more than 15 minutes, the DNA will break down. Slowly stir the mixture periodically but not so much as to create foam. Make sure to record the time you placed the beaker or flask in the heating environment.

4. After 10 to 12 minutes, remove the solution from the hot water bath or hot plate and place it in the ice water bath for five minutes. Stir the solution slowly while it is cooling.

5. Filter the solution into a plastic cup using the funnel or strainer and coffee filter. Try to avoid getting any foam into the filtered mixture. This filtering procedure may be a slow process; if needed, it can be left to filter overnight in a refrigerator.

6. Divide the filtered solution into the test tubes, placing about one teaspoon in each. Stir the filtered solution while dividing it. The test tubes can be stored in the refrigerator overnight.

7. Add two toothpicks full of meat tenderizer to the solution in the test tubes and **gently** mix. Next, add **cold** alcohol to the solution until a 1 cm layer of alcohol forms on top of the solution. This can be done by using a medicine dropper or pipette. **Do not mix the solution!** Watch as the DNA begins to precipitate out into the alcohol. The bent piece of glass tubing can be used to spool the DNA. The DNA will look like white mucus.

Key Questions:

1. What did the soap do to aid in the extraction of the DNA?

2. Why was the onion solution heated and then cooled?

3. Why was meat tenderizer used? (Hint: Look at the ingredients in the meat tenderizer.)

4. Why did the DNA precipitate out in the alcohol?

5. Do you think the DNA obtained was pure? Why or why not?