DNA EXTRACTION

Unit: Animal Science

Problem Area: Animal Genetics and Biotechnology

Lesson: DNA Extraction

Student Learning Objectives. Instruction in this lesson should result in students achieving the following objectives:

1. **Explain the genetic information found in cells.**
2. **Explain the DNA molecule.**
3. **Explain how DNA is copied.**
4. **Understand how genes code for a single protein.**

List of Resources.

The following resources may be useful in teaching this lesson:

Baker, Meecee and Mikesell, Robert E. *Animal Science Biology & Technology*. Danville, IL: Interstate Publishers, Inc., 1996 (Chapter 6).

Ensminger, M.E. *Animal Science.* 9th Edition. Danville, IL: Interstate Publishers, Inc., 1991 (Chapter 3).

Gillespie, J.R. (2002) *Modern Livestock & Poultry Production* (6th Edition). Albany, NY: Delmar. (Unit 9)

Lee, Jasper S., et al. *Introduction to Livestock & Companion Animals.* 3rd Edition. Upper Saddle River, NJ: Pearson Prentice Hall Interstate, 2004.

Lee, Stephen J., et al. *Biotechnology*. Danville, IL: Interstate Publishers, Inc., 2001 (Chapter 10).

List of Equipment, Tools, Supplies, and Facilities

- ✓ Writing surface
- ✓ Overhead projector
- ✓ Transparencies from attached masters
- ✓ Copies of student lab sheets
- ✓ Copies of technical supplement

Terms.

The following terms are presented in this lesson (shown in bold italics):

- Adenine
- Chromosomes
- Cytosine
- DNA
- Double Helix
- Eukaryotic
- Gene Expression
- Genes
- Genetic Code
- Guanine
- Messenger RNA
- Nucleotides
- Prokaryotic
- Proteins
- Replication
- RNA
- Ribosomal RNA
- Thymine

- Transcription
- Transfer RNA
- Translation

Interest Approach. Use an interest approach that will prepare the students for the lesson. Teachers often develop approaches for their unique class and student situations. A possible approach is included here.

> *Obtain a copy of the first Jurassic Park movie. View the segment in the movie where they discuss chromosomes, DNA, and genes. The clip explains how a scientist found a fossilized mosquito that had sucked dinosaur blood, preserving the dinosaur DNA. The scientist then explains how frog DNA was used to complete the missing dinosaur DNA code to create a new dinosaur. Lead to a discussion on whether or not this is possible. Let this lead to a discussion on DNA and DNA extraction.*

SUMMARY OF CONTENT AND TEACHING STRATEGIES

Objective 1: Explain the genetic information found in cells.

Anticipated Problem: What structures responsible for genetic information are found in cells?

I. Genetic information is important in developing cells.

A. There are many structures that are responsible for genetic information. Inside cells are chromosomes, DNA and genes. ***Chromosomes*** are rod shaped structures that carry genetic material. ***DNA*** or deoxyribonucleic acid is a long thin molecule that determines the cell's characteristics. Some of the DNA information is organized into ***genes***, which are segments of DNA that transmit information from parent to offspring.

B. When genes are being used the DNA is extended so other molecules can retrieve the information. When the cell is ready to reproduce, protein is added to the DNA and it is coiled and twisted into a dense chromosome.

> *Achieving this objective should focus on clarifying the meaning of chromosomes, DNA, and genes. Students may be called on to explain the meaning of the terms and how the terms fit together. Use TM–A to help reinforce the idea that the genetic material is found inside a cell. Use TM–B and TM–C to show the students where the genetic material is found within the cell.*

Objective 2: Explain the DNA molecule.

Anticipated Problem: What is DNA?

II. DNA is a long thin molecule made of subunits called nucleotides that are linked together in a chain.

A. ***Nucleotides*** are subunits of DNA that are made of a nitrogen base, a 5 carbon sugar and a phosphate group. The 5 carbon sugar is called deoxyribose and is the same in all DNA. The phosphate group is also the same in all DNA. However, the nitrogen base can be different. It can be ***adenine***, ***guanine***, ***thymine*** or ***cytosine***.

B. DNA is found in a double helix. A ***Double helix*** is a spiral staircase of 2 strands of nucleotides twisting around a central axis. A double helix looks like a twisted ladder. The sides are alternating 5 carbon sugars and phosphates. The rungs are nitrogen bases held together by hydrogen bonds.

C. The nitrogen bases are found in pairs. Adenine can only form a hydrogen bond with thymine. Guanine can only form a hydrogen bond with cytosine. The nitrogen bases found on one strand determines the nitrogen bases found on the other strand.

The overall goal of this objective is to explain the structure of DNA and how the nucleotides are linked together. TM–D should be used to explain the double helix formation as well as the position if the four nitrogen bases.

Objective 3: Explain how DNA is copied.

Anticipated Problem: How is DNA copied?

III. DNA is copied by separating the strands and using one strand as a template.

A. ***Replication*** is the process of making a new strand of DNA.

1. Enzymes unwind the DNA by breaking the hydrogen bonds.
2. New nucleotides are added to the exposed nitrogen bases.
3. Nitrogen bases are paired correctly.

TM–E can be used to summarize the general areas of the objective.

Objective 4: Understand how genes code for a single protein.

Anticipated Problem: How do genes code for a single protein?

IV. Genes hold the information about how to make a specific protein.

A. ***Proteins*** are what affect how an organism looks. Proteins are not built directly from the genes. The genes are changed into RNA and the RNA is changed into proteins. ***RNA*** or ribonucleic acid instructs genes to build proteins.

1. RNA differs from DNA in 3 ways. RNA has only one strand of nucleotides, the name of RNA's five carbon sugar is ribose, and the nitrogen bases are adenine, guanine, cytosine and uracil.
2. There are five types of RNA and each has a different job in changing genes into proteins. ***Messenger RNA*** or mRNA is a copy of a gene that is used as a blueprint for a protein. When a cell needs a particular protein it makes that specific mRNA. ***Ribosomal RNA*** or rRNA translates mRNA into amino acids to make proteins. ***Transfer RNA*** or tRNA contains the anticodon for the ***genetic code,*** which is the code that stores information for all genetically determined characteristics.

B. ***Gene expression*** is the process of changing the genes in DNA into proteins. There are two steps to gene expression. ***Transcription*** is when the information from the genes in DNA is changed to mRNA. ***Translation*** is when the information in mRNA is changed into amino acids used to make protein. In ***prokaryotic*** organisms which are organisms without a membrane around its genetic material, transcription occurs in the cytoplasm. In ***eukaryotic*** organisms or organisms with a membrane around its genetic material found in a structure called the nucleus, transcription occurs in the nucleus.

This objective explains how genes code for a single protein. Use TM–F to understand the similarities and differences between DNA and RNA.

Review/Summary. Use the student learning objectives to summarize the lesson. Have students explain the content associated with each objective. Student responses can be used to determine which objectives need to be reviewed or re-taught.

Application. Use the following transparency masters, lab sheets, and technical supplement to apply the information.

- TM–A: The Animal Cell
- TM–B: Chromosomes, DNA, and Genes
- TM–C: The Structure of DNA
- TM–D: DNA Structure
- TM–E: DNA Replication
- TM–F: DNA and RNA

- LS–A: DNA Extraction—Calf Thymus
- LS–B: DNA Extraction—Cheek Cells
- TS–A: DNA Extraction

Evaluation. Evaluation should be based on student comprehension of the learning objectives. This can be determined using the attached sample test.

Answers to Sample Test:

Part One: Matching

1. h
2. b
3. f
4. a
5. i
6. d
7. c
8. g
9. e

Part Two: Fill-in-the-Blank

1. RNA
2. Three
3. Gene expression
4. Deoxyribonucleic acid
5. Adenine, Guanine, Thymine, Cytosine

Part Three: Multiple Choice

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

Part Four: Short Answer

1. Chromosomes contain DNA and genes are found of segments of DNA.
2. In pairs
3. Genetic code

Test

Name _____

DNA EXTRACTION

▶ Part One: Matching

Instructions: **Match the word with the correct definition.**

a. DNA	d. Nucleotides	g. Proteins
b. Chromosomes	e. Double Helix	h. Eukaryotic
c. Genes	f. Replication	i. Prokaryotic

_____1. Organism with a membrane around its genetic material.

_____2. Rod shaped structures that carry genetic material.

_____3. The process of making a new strand of DNA.

_____4. Long thin molecule that determines the cell's characteristics.

_____5. Organisms without a membrane around its genetic material.

_____6. Subunits of DNA that are made of a nitrogen base, a 5 carbon sugar and a phosphate group.

_____7. Segments of DNA that transmit information from parent to offspring.

_____8. What affects how an organism looks.

_____9. A spiral staircase of 2 strands of nucleiotides twisting around a central axis.

▶ Part Two: Fill-in-the-Blank

Instructions: **Complete the following statements.**

1. _____ instructs genes to build proteins.
2. There are _____ types of RNA.
3. _____ _____ is the process of changing the genes in DNA into proteins.
4. DNA stands for _____ _____.
5. _____, _____, _____, and _____ are the four nitrogen bases.

▶ Part Three: Multiple Choice

Instructions: **Circle the letter of the correct answer.**

_____1. DNA is made up of _____.

a. A nitrogen base
b. A phosphate group
c. 5-carbon sugar
d. All of the above

_____2. DNA is a long thin molecule made of subunits called _____.

a. Proteins
b. Nucleotides
c. Carbon
d. Chromosomes

_____3. All of the following are nitrogen bases except

a. Adenosine
b. Guanine
c. Thymine
d. Cytosine

_____4. The _____ is called deoxyribose and is the same in all DNA.

a. 5-carbon sugar
b. 3-carbon sugar
c. Phosphate group
d. None of the above

▶ Part Four: Short Answer

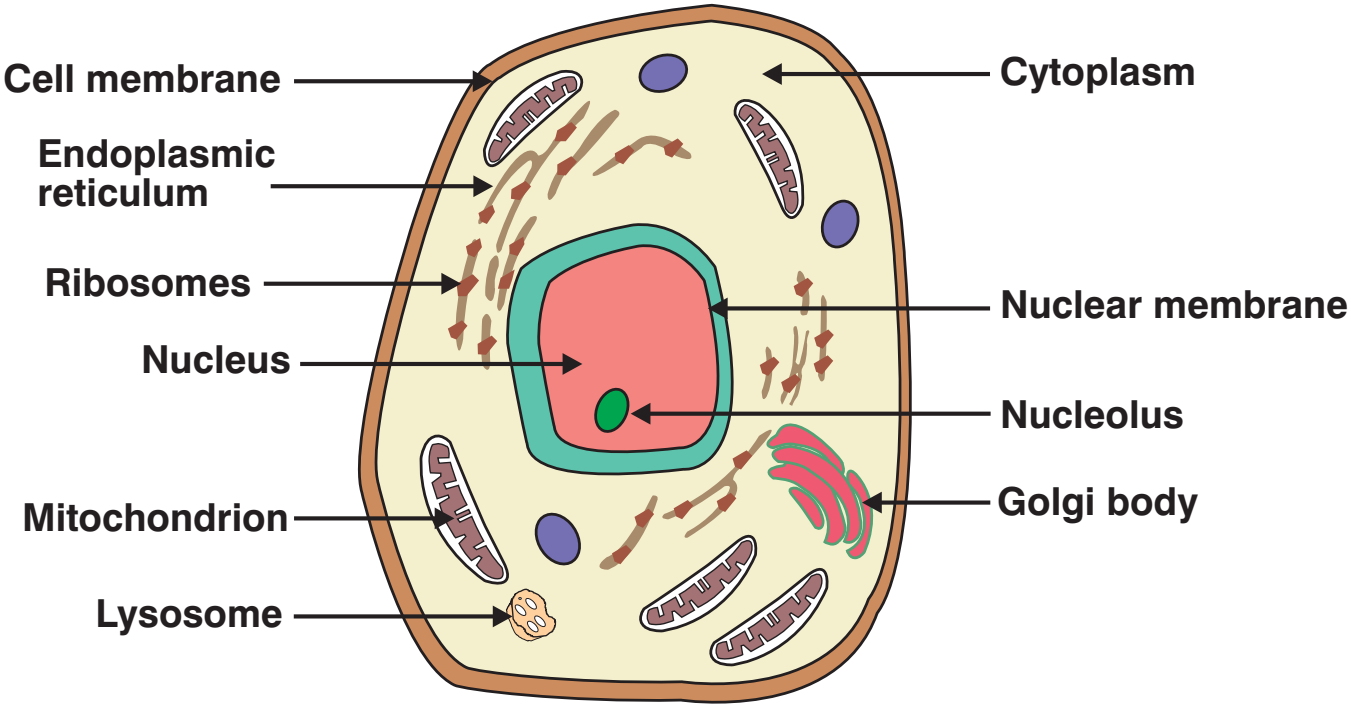
Instructions: **Answer the following questions.**

1. How do chromosomes, DNA, and genes relate to one another?

2. How are nitrogen bases found?

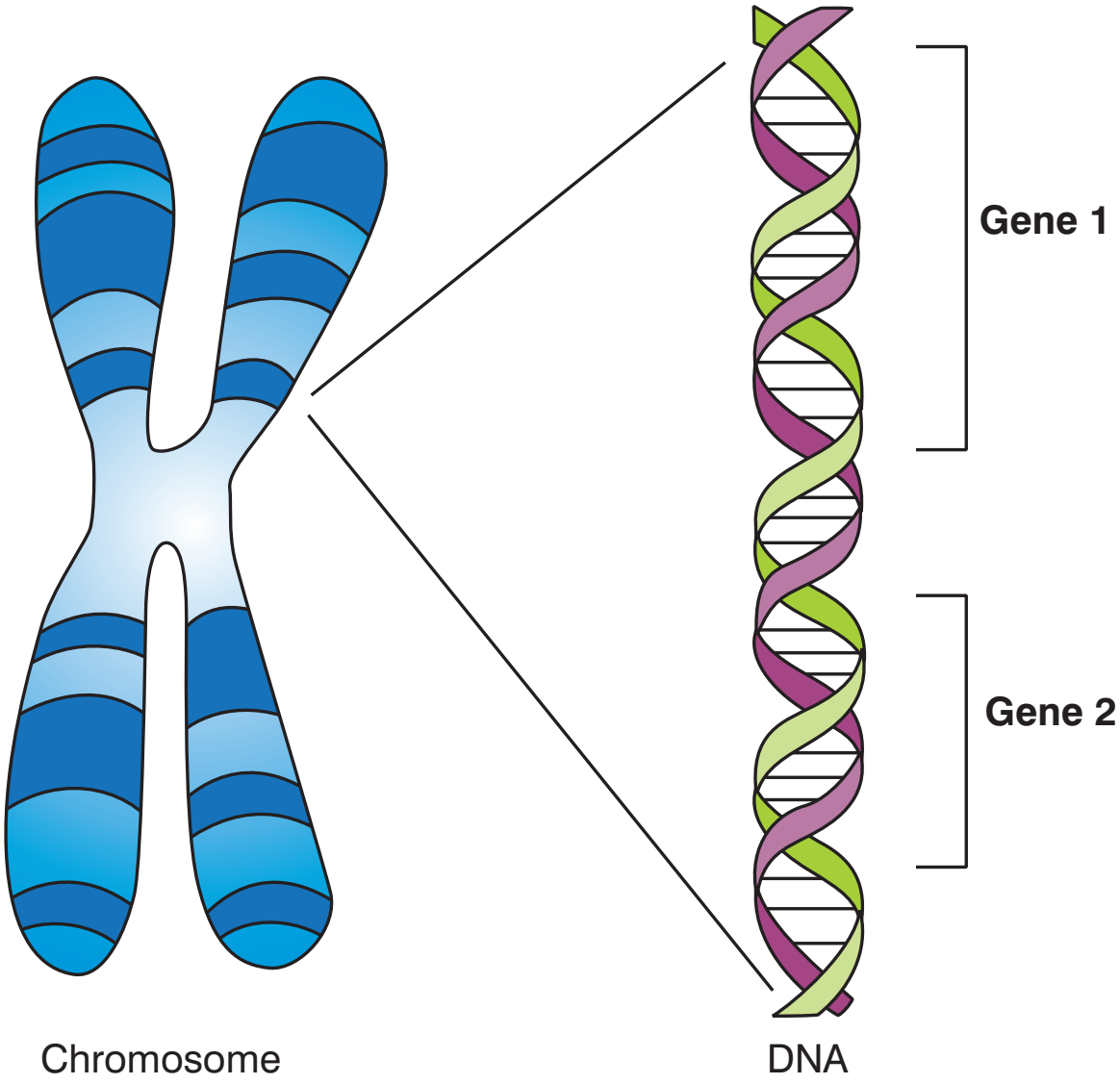
3. The code that stores for all genetically determined characteristics is known as what?

THE ANIMAL CELL

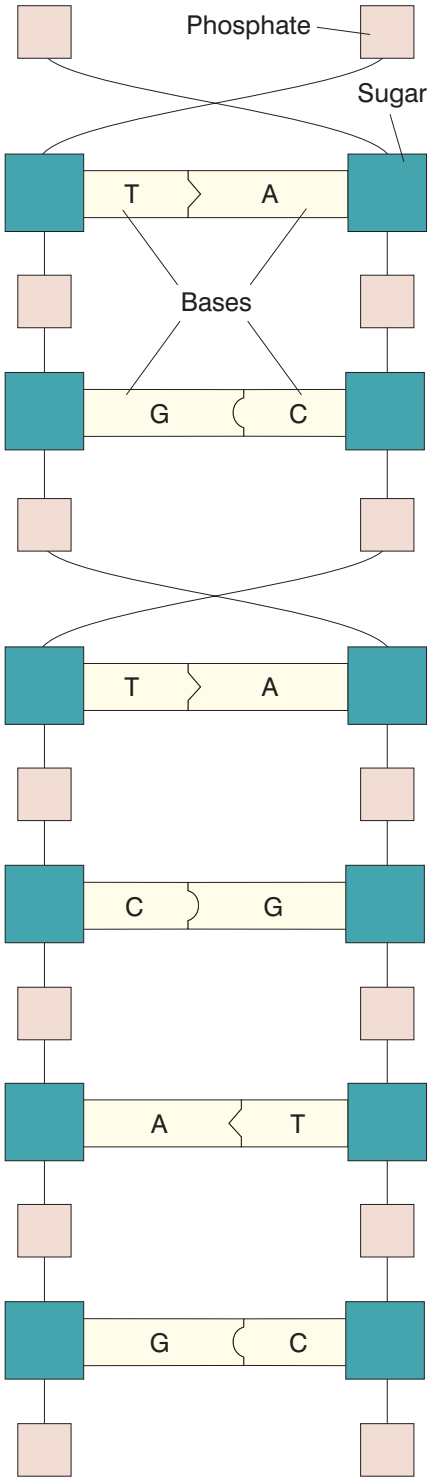


TM–B

CHROMOSOMES, DNA, AND GENES

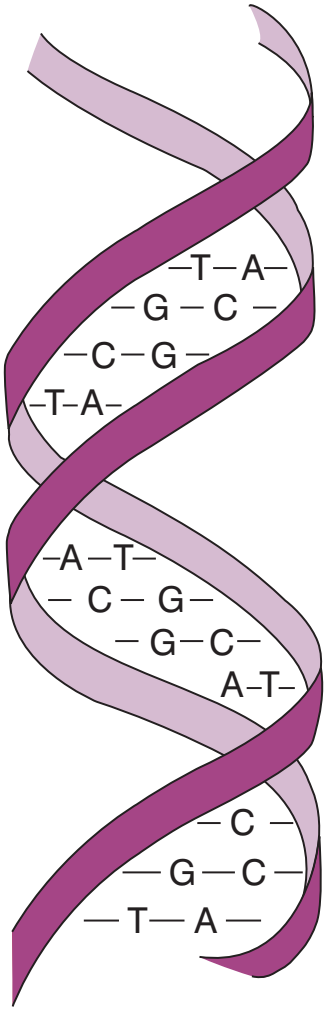


THE STRUCTURE OF DNA

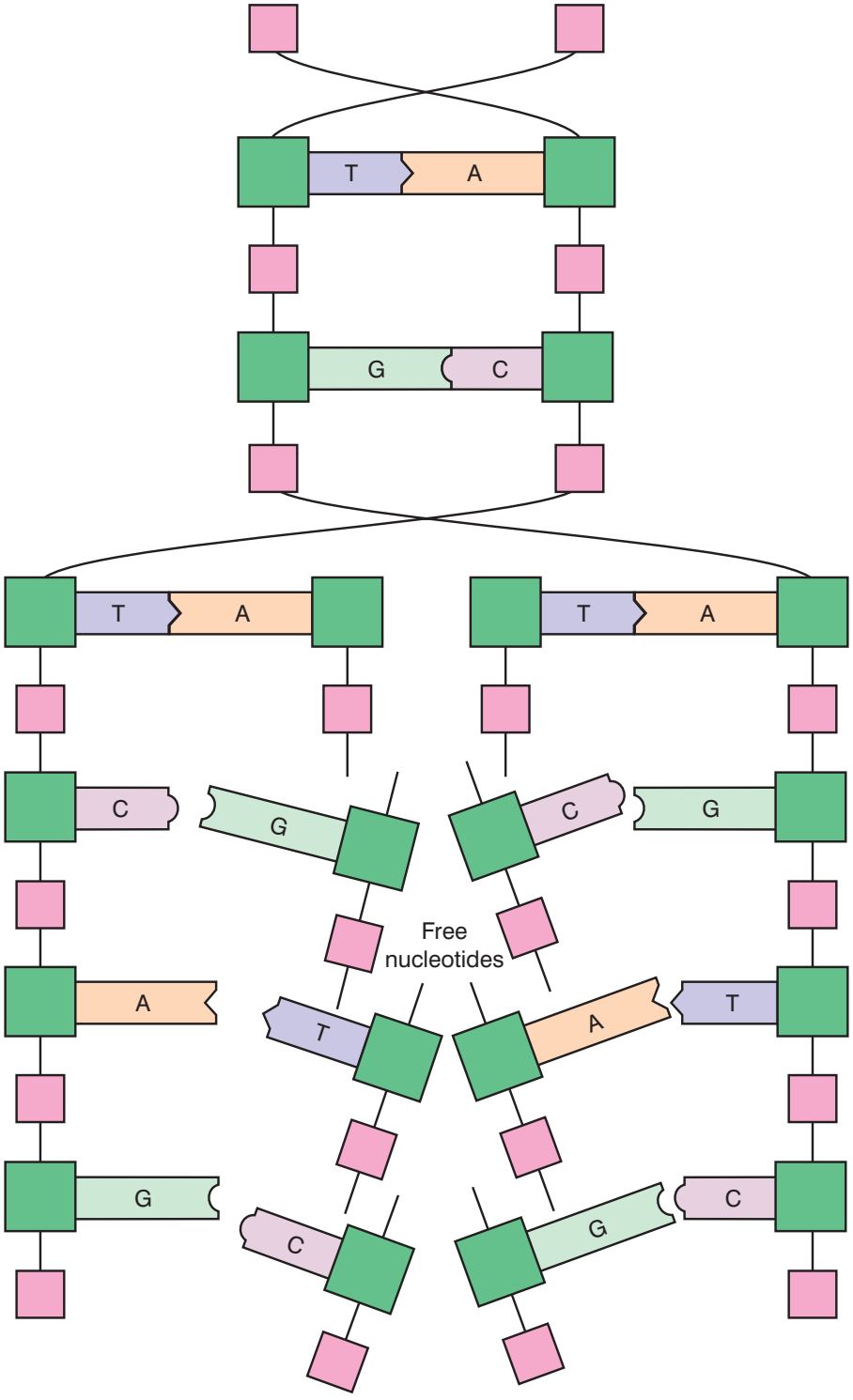


DNA STRUCTURE

- Chromosomes are made of genes that consist of DNA. DNA is a protein-like nucleic acid on genes that controls inheritance.
- Each DNA molecule consists of two strands shaped as a double helix
- There are four nitrogen bases found in DNA. They are: cytosine, guanine, adenine, and thymine.
- The genetic code is the sequence of nitrogen bases in the DNA molecule. Replicating itself allows for the molecule to pass genetic information from one cell generation to the next.



DNA REPLICATION



DNA AND RNA

- RNA differs from DNA in three ways:
  - RNA has only one strand of nucleotides.
  - The name of RNA's five carbon sugar is ribose.
  - The nitrogen bases are adenine, guanine, cytosine and uracil.
- Messenger RNA (mRNA)—a copy of a gene that is used as a blueprint for a protein.
- Ribosomal RNA (rRNA)—translates mRNA into amino acids to make proteins.
- Transfer RNA (tRNA)—contains the anticodon for the genetic code.
- Gene expression—the process of changing the genes in DNA into proteins.
  - Transcription is when the information from the genes in DNA is changed to mRNA.
  - Translation is when the information in mRNA is changed into amino acids used to make protein.

DNA EXTRACTION—CALF THYMUS

Agriculture Applications and Practices

Farmers and ranchers throughout the world have long tried to improve the organisms with which they work. By selectively breeding the most productive animals to produce the next generation, people have found that the productivity of a domesticated species can gradually be increased. Due to technological advancements, we no longer have to wait for breeding methods to produce better animals. This can be achieved by altering the DNA of a particular organism. DNA or deoxyribonucleic acid is the molecule that carries genetic information. By manipulating this genetic information, scientists are able to produce animals that are leaner, more heavily muscled and more productive.

Scientists have determined a way to take a gene from one organism and place it into another. This can be accomplished by cutting the DNA containing the gene wanted away from the genes surrounding it, combining the gene with a piece of DNA from the recipient organism, inserting the combined DNA into the organism, and finally reading the sequences of nucleotide bases in the gene in order to analyze the genes that are being manipulated. This technique is currently being used and will continue to advance to help engineer animals that are bigger, faster growing and more efficient.

Science Connections—Questions for Investigation

1. What is the basic structure of DNA?
2. How is information stored within the DNA structure?
3. How can DNA be isolated?
4. What is the basic function of DNA?

Research Problem

What are the physical and chemical properties of DNA?

Purpose of the Laboratory and Students Performance Objectives

The purpose of this experiment is to investigate the basic properties of DNA. Through this laboratory exercise and related discussion, students will be able to:

1. Extract a visible mass of DNA.

2. Describe the physical and chemical properties of DNA.
3. Explain the universal function of DNA.

Introduction

The thymus is a gland that is very large in immature animals. It functions as part of the immune system so there are many white blood cells with large nuclei present. Many thousands of thymus cells will be used for this extraction so you will be combining thousands of nuclei. In this way, you should be able to see long, combined strands of DNA.

Material

- 0.9% NaCl (0.9g of NaCl in 100 ml distilled water
- 10% dish washing liquid (10ml in 90 ml water)
- 95% ethanol
- mortar and pestle
- test tubes
- scissors and/or scalpel
- cheese cloth
- glass stirring rod
- calf thymus (sweetbread)
- small graduated cylinder

Procedure

Give each student or group of students a copy of the worksheet to perform the activity.

Helpful Hints

- The precipitated DNA can be stored by transferring it to a fresh test tube or container containing 95% ethanol. Freeze indefinitely.

Anticipated Findings

- Make sure that students understand that they will not be looking at one DNA molecule on the spooling rod; instead several strands of DNA will be coiled together. Students are sometimes under the impression that they will observe a single double helix and this is simply not possible.

Name _____

DNA EXTRACTION—CALF THYMUS

Procedure

1. Place a small piece of calf thymus (sweetbread from the local butcher) into a mortar. Use about a cubic centimeter. Cut the sample into smaller pieces using a scalpel or scissors.
2. Add 10 ml. of 0.9% sodium chloride solution (NaCl), and grind with the pestle for about 2–5 minutes.
3. Strain the solution through three or four layers of cheesecloth (available from your local fabric shop) into a test tube. Throw away the cheesecloth and keep the suspension.
4. Add 1.5 ml of 10% dish washing liquid (Dawn). 10 ml detergent in 90 ml water. The detergent lyses the cell membrane just like it removes grease from dishes.
5. Measure the total sample volume, then measure out two times that amount of ice cold 95% ethanol. Tilt your test tube and gently add the ethanol to the suspension by pouring the ethanol down the side of the test tube.
6. Using a clean, glass stirring rod, gently stir the mixture at the interface until the DNA begins to precipitate. It may start to precipitate as soon as you add the ethanol. Then twist the glass rod to spool the DNA sample onto the rod.

DNA EXTRACTION—CHEEK CELLS

Purpose of the Laboratory and Students Performance Objectives

The purpose of this experiment is to investigate the basic properties of DNA. Through this laboratory exercise and related discussion, students will be able to:

1. Extract a visible mass of DNA.
2. Describe the physical and chemical properties of DNA.
3. Explain the universal function of DNA.

Introduction

Can DNA be found in human eukaryotic cells? DNA, a polar molecule composed of two complementary chains of nucleotides wound in a double helix, is present in all living things from bacteria to plants to animals. In animals, it is found in almost all cell types: muscle fibers, reproductive cells, white blood cells, and skin cells. The basic procedure for extracting DNA is the same, regardless of its source, although the specifics may vary:

- Collect the cells containing DNA
- Break the cellular membranes to release the DNA
- Separate the cellular components from the DNA
- Precipitate the molecules of DNA

Materials

- 8 grams sodium chloride (not ionized) "i.e. canning salt"
- 25 mL plain clear liquid soap
- 10 mL "cooled" isopropyl alcohol 70%+
- 200 mL of distilled water
- 10 mL of bottled drinking water
- 2 250 mL glass beakers
- 1 100 mL graduated cylinder
- 1 25 mL graduated cylinder

- 1 electronic balance
- 1 eyedropper
- 1 paper cup
- 1 test tube
- 1 test tube stopper/lid
- 1 test tube rack
- 1 spoon
- 1 glass-stirring rod
- 1 toothpick (optional)

Procedure

Give each student or group of students a copy of the worksheet to perform the activity.

Helpful Hints

- Soap and saline solutions may be used for the entire class.

Name _____

DNA EXTRACTION—CHEEK CELLS

1. Clean your mouth of any residual food items by rinsing your mouth with drinking water prior to the next step.
2. Using a 25 mL graduated cylinder, measure and pour 10 mL of the bottled drinking water into a paper cup. Swirl the drinking water in your mouth for about 3–4 minutes; it would help greatly if you also firmly, yet gently scraped the inside of mouth with your teeth to insure a high cell count.
3. Slowly relinquish the "mouthwash" solution back into the paper cup. Carefully pour the entire contents of the "mouthwash" solution into a test tube and set aside.
4. Using a 100 mL graduated cylinder, measure 75 mL of distilled water. Place the distilled water into a 250 mL beaker. Using the same graduated cylinder, measure and place 25 mL of liquid soap into the beaker with the distilled water. Gently stir the "soap solution" with a clean stirring rod until the solution is well mixed with few suds.
5. Using an eyedropper, place 1 dropper "full" of liquid soap solution from the beaker into the test tube containing the "mouthwash" solution and set aside.
6. Create an 8% sodium chloride solution by doing the following: Using a 100 mL graduated cylinder, measure 92 mL of distilled water. Place the water into the other 250 mL beaker. Measure and place 8 grams of sodium chloride into the beaker with the distilled water. Stir the "saline solution" with a clean stirring rod until the salt is totally dissolved.
7. Using an eyedropper, place 1 dropper "full" of the saline solution from the beaker into the test tube containing the "mouthwash" and "soap" solution.
8. Cover the top of the test tube with a stopper and while holding your thumb on the stopper, gently mix the contents by turning the test tube upside down and right side up about 10 times.

9. Using an eyedropper, place 5 dropper "fulls" of "cooled" 70%+ isopropyl alcohol into the test tube containing the mouthwash, soap, and saline solution. Make sure to pour it at an angle down the side of the test tube. It is highly important that no sudden movements of the solution be performed during this time.

10. Place test tube in a test tube rack and wait for about 3–5 minutes for the DNA to precipitate and float to the surface. Using a stirring rod or toothpick gently "swirl" the DNA on to the tool.

Technical Supplement

DNA EXTRACTION

1. What is the basic structure of DNA?

DNA (deoxyribonucleic acid) is found in the cells of all living things and is universal to all living organisms. It is transferable from one organism to another. DNA has a structure that resembles a long, uncooked strand of spaghetti, and while appearing to be flexible, is strong, stiff, and will break if bent too far. In 1953 Francis Crick and James Watson proposed a model of the DNA structure as a double helix spiral of a shape comparable to a twisted rope ladder. DNA is composed of many building blocks called nucleotides, which consists of nitrogen containing organic base, five carbon (pentose) sugar, and a phosphate group. The sides of the DNA "ladder" are composed of alternating sugar and phosphate groups while the "ladder" rungs consist of the nitrogen bases.

Imagine this ladder split into two parts lengthwise. Each part would contain a side with many half rungs. The complete DNA ladder is formed by joining the half rungs (nucleotides) of the two sides with weak hydrogen bonds. There are four types of DNA nucleotides. Two of these, called adenine and guanine, are known as purines while the other two, called thymine and cytosine, are known as pyrimidines. These half rungs of the ladder will bond in only two combinations: adenine to thymine and guanine to cytosine. These nucleotides are part of a code system that controls protein synthesis in the cell. There may be as many as several million nucleotides in a single DNA strand, which is said to be three meters long in humans. It is the different variations in arrangements of these four nucleotide bases on the DNA ladder, which differentiates the various species of living things.

2. How is information stored within the DNA structure?

Within the nucleus of an individual cell are rod shaped structures called chromosomes that are made up of DNA. A small segment of this DNA is called a gene. Each

gene occupies a specific location on the DNA strand and provides a blueprint code for the synthesis of a single, specific protein within the cell. In addition, these genes transfer these hereditary codes when cells reproduce. A single gene may be made up of as many as a thousand nucleotide pairs.

3. How can DNA be isolated?

In recent years, biotechnology techniques have allowed us to extract and isolate DNA to identify the location of individual genes. This is called gene mapping. Once mapped, genes can be transferred from one organism to another. This recombinant DNA allows for changing the genetic makeup of the organism. DNA can be extracted from cells and its physical and chemical properties may be studied. A detergent is first used to dissolve cell membranes. This will cause the cell contents to become suspended in solution. This solution is heated to prevent enzymes from breaking down the DNA molecule. Alcohol is then added to the contents to bring the DNA out of solution as a precipitate. A glass rod can then be used to spool or wind floating DNA for observation.

Another method of isolating DNA is through electrophoresis. Enzymes are used to cut the DNA molecule into smaller segments. A sample of this material is placed on an agarose gel in a buffer solution. As electrical current is applied to the solution, the DNA segments, which are negatively charged due to the phosphate groups, are attracted to the positive side of the electrical field. As the segments migrate, the smaller pieces are attracted faster and the segments become sorted by size for further study and mapping. This process is referred to as Restriction Fragment Length Polymorphisms (RFLP) and is useful in such areas as DNA "finger printing."

A third method of DNA isolation is called the DNA probe. A probe is a segment of DNA from one cell that can combine with an identical gene of another cell. The process is compared to fishing, where the DNA probe is used like a hook to fish among the genetic material for its complementary DNA.

4. What is the basic function of DNA?

Cells are fundamental units of life for all living things. Cells form the structure of organisms and contain the hereditary information for new cells as they reproduce. Every cell contains and manufactures proteins. The living condition of an organism depends upon the exact and correct kinds of protein in each cell. A slight difference in cell protein has a major effect upon the shape and function of the cell. Cells copy DNA for instructions on combining amino acids into proteins. The DNA transcribes theses instructions to RNA in the cytoplasm where proteins synthesis takes place. DNA also functions in transferring the hereditary traits of parent cells to daughter cells during mitosis and meiosis.