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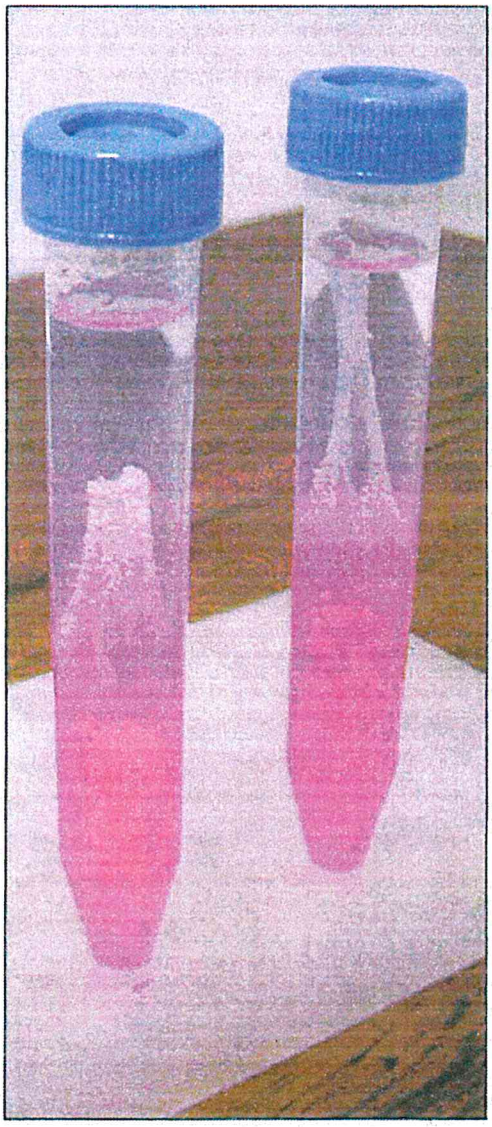
DNA Necklace Kit

TEACHER'S MANUAL



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DNA Necklace Kit
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Student Guide S-1

DNA Necklace Kit

Overview

Using this kit, students isolate their own human genomic DNA and create wearable DNA necklaces. Each student follows a simple procedure to extract crude DNA from his or her own cheek cells. During the procedure, students lyse a sample of their cheek cells and watch as wispy white strands of their own chromosomal DNA precipitate out of solution in the presence of ethanol. Each student transfers their DNA to a plastic microcentrifuge tube and fashions the tube and a colored string into a DNA pendant necklace.

Objectives

- Students will learn the function of DNA.
- Students will learn how DNA is contained within cells.
- Students will learn how DNA can be isolated from cells by performing a DNA extraction from their own cheek cells.

Background Information

DNA Is the "Code of Life"

DNA can be considered the hereditary "code of life" because it possesses the information that determines an organism's characteristics and is transmitted from one generation to the next. DNA can be compared to a recipe or a list of instructions about how to create and maintain a specific living thing. Almost all structure and function of living things is dependent on and determined by DNA. In addition, the structure of DNA is consistent among all species.

However, genes (sequences that occupy specific locations on DNA and control particular traits) differ between organisms. This creates explicit "blueprints" for building individual living things. Genes encode the information to make proteins and determine how many of those proteins will be made. For example, our DNA controls the pigment color of our skin. Each human skin cell produces a specific amount of melanin protein (pigment) according to the DNA instructions of the melanin gene. Inherited variations in this gene account for skin color variation in the human population.

Packaging of DNA

DNA is contained within the nucleus of almost every cell in the human body. The length of DNA per cell is about 100,000 times as long as the cell itself. However, DNA takes up only about 10% of the cell's volume. This is because DNA is specially packaged through a series of compaction events to fit easily within cell nuclei (see Figure 1).

The basic structure of DNA is that of a twisted ladder called a double helix. In order to condense the length of this DNA molecule, the double helix wraps itself around groups of histone proteins. Histone proteins wrapped by DNA look like beads on a string. This complex of DNA and histone proteins is called chromatin and the bead-like regions of histones and wrapped DNA are called

nucleosomes. To further condense the DNA, the chromatin then folds back on itself and the nucleosomes pack together to create a compact, protein-coated fiber. The fiber coils to shorten further into an extended chromosome. At its most compressed state, this coiled fiber organizes into loops emanating from a central axis (scaffold) to create a condensed, X-shaped chromosome (see Figure 1).

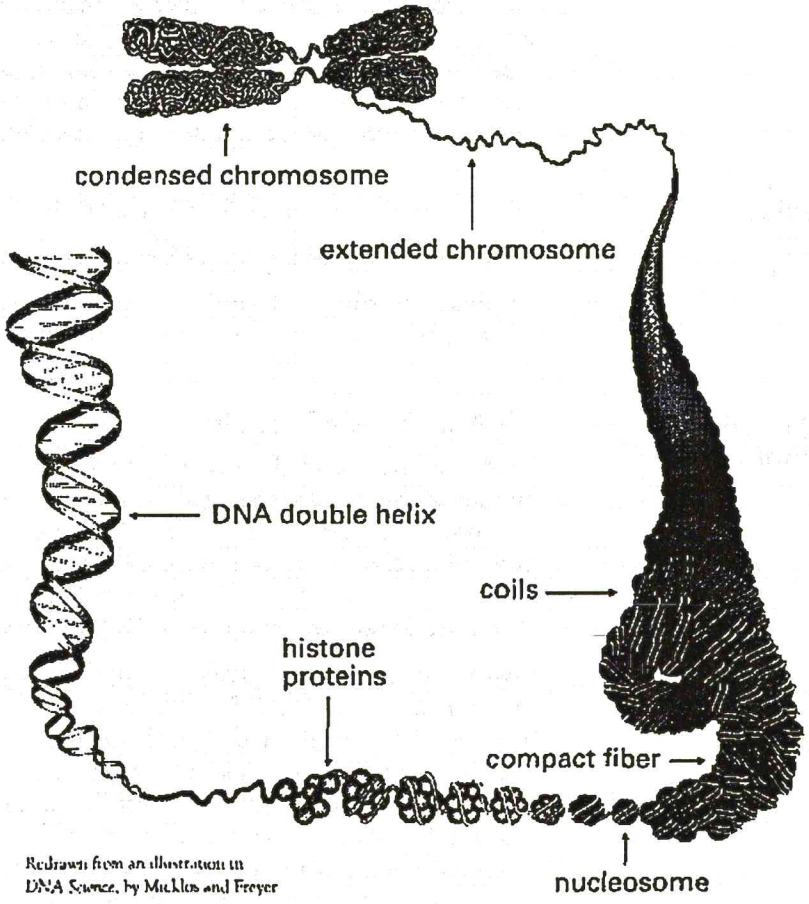

Figure 1. Condensation of double-helical DNA into a chromosome

Human beings have 46 chromosomes in the nucleus of almost every cell. As described above, each chromosome is comprised of one long continuous stretch of DNA that has been compacted and folded around proteins. If the 46 chromosomes from one human cell were unraveled and lined up end-to-end, they would measure approximately 6 feet long! When cells are not dividing (in interphase), the chromosomes are extended and bunch together, filling the nucleus like a ball of cotton. During cell division (mitosis) the chromosomes condense and become distinct as 46 individual X-shaped structures (see Figure 1).

Chromosomal DNA from a single cell is not visible to the naked eye. However, when chromosomal DNA is extracted from multiple cells, the amassed quantity can easily be seen and looks like strands of mucous-like, translucent cotton. This DNA is coated with proteins and is at various stages of condensation (see Figure 1). Individual condensed chromosomes can be

folded and condensed around protein molecules and is considered crude because it has not been separated from the cellular proteins that adhere to it during precipitation. The DNA double helix is masked by the protein molecules and exists at the molecular level. Therefore, the helix cannot be seen by eye or even with common microscopes.

Importance of DNA Extraction

The principles of DNA extraction used in this activity apply to procedures used by scientists to extract DNA from a multitude of sources. DNA extraction is a fundamental procedure in scientific laboratories around the world and is typically the first step in many in-depth laboratory experiments. By extracting DNA and studying it, scientists can learn how DNA encodes the instructions for all life processes. DNA extraction is important to the study of heredity and to the potential treatment of diseases through the creation of gene therapy DNA molecules. Extracted DNA can also be used to create DNA fingerprints to help diagnose genetic diseases, solve criminal cases, identify victims of disaster and war, and establish paternity or maternity. Scientists can genetically engineer changes in DNA to create robust, disease-resistant, genetically modified plants and animals. DNA extraction is also necessary in order to sequence and compare the DNA code of different organisms (as in the Human Genome Project).

Time Requirements

The DNA Necklace classroom activity should take approximately 45–60 minutes to complete. The following amount of time is required for each step of the procedure:

Time	Activity
20 minutes	Teacher preparation
5 minutes	Cheek cell collection
10–30 minutes	Optional cell pelleting step
20 minutes	Cheek cell lysis and addition of ethanol to cell lysate
10 minutes (minimum)	DNA precipitation
10–15 minutes	DNA transfer and necklace assembly

studied using microscopes, but the double helix of a chromosome is so thin that it can be detected only by innovative techniques, such as three-dimensional computer imaging of DNA molecular measurements and scanning probe microscopy.

DNA Extraction From Human Cheek Cells

In order to extract chromosomal DNA from human cheek (epithelial) cells, a sample of intact cheek cells must first be collected. In this activity, a sports drink is used as a mouthwash collection medium. This is because sports drinks contain salt (sodium chloride), and thereby are saline solutions compatible with the osmotic environment of the cells. That is, the cheek cells do not dramatically gain or lose volume during collection with this saline rinse. If water were used as the mouthwash collection medium instead of a saline solution, it would be hypotonic in comparison to the cells, causing them to flood with water (by osmosis) and burst. The sports drink prevents the cells from breaking open and releasing their DNA before they are collected. This method of collection amasses a large quantity of cheek cells from which DNA is extracted.

Once the intact cells are collected, the membrane barriers that surround the cells and their nuclei must be broken. These membranes act to protect and separate the cell, organelles, and DNA from the surrounding environment. Cell membranes and nuclear membranes are comprised of two layers of phospholipids called a phospholipid bilayer. The process of breaking open the membranes of a cell is called cell lysis. In this activity, the cell membranes and nuclear membranes are disrupted with a detergent-based cell lysis solution. Just as a dishwashing detergent dissolves fats (lipids) to cleanse a frying pan, a detergent-based cell lysis solution dissolves the phospholipid bilayer of cell membranes by forming water-soluble complexes with them. Once the cell membranes are degraded, the cell contents flow out and create a soup of dissolved membranes, cellular proteins, DNA, and other contents. This "soup" is called the cell lysate.

The DNA in the cell lysate is in solution. This means that it is incorporated in the liquid lysate and is not visible (like a teaspoon of sugar in a cup of water). However, DNA is insoluble in ethanol (an alcohol), meaning that it cannot be incorporated into the liquid (like a teaspoon of sand in a cup of water). Therefore, DNA from the cell lysate can be visualized by applying a layer of ethanol on top of the cell lysate.

Once the ethanol hits the cell lysate, it causes the DNA to precipitate out of the solution, forming a translucent cloud of fine, stringy fibers at the point where the ethanol and cell lysate meet. Cold ethanol works best to precipitate DNA to the fullest. DNA is negatively charged due to the phosphate ions of its backbone. Positive sodium ions from the salt in the sports drink are attracted to the negatively charged DNA and thereby neutralize its charge. This neutralization counteracts the repulsion of the DNA strands and allows them to clump together as they precipitate.

It is important to remember that the extracted DNA from the human cheek cells represents a huge collection of chromosomes (many strands of DNA) isolated from a large quantity of cells and clustered together. This DNA is

Instructions

1. Using a marker or label provided by your teacher, write your name or initials on your 15-mL tube.
2. Pour the sports drink from the small plastic cup into your mouth (do not swallow) and swish it around for 1 full minute. As you swish, gently and continuously scrape the insides of your cheeks with your teeth to help release the cheek cells.
3. After 1 minute of swishing, spit the sports drink with collected cheek cells back into the small plastic cup.
4. Carefully pour the contents of the small plastic cup into your labeled 15-mL tube. Discard the cup.
5. *This is an optional step that will not affect the results of the procedure but allows collected cheek cells to be visualized. You may be instructed to perform this step or to skip ahead to Step 6.*

   If time allows, you can watch your collected cheek cells settle out as a pellet in the bottom of your 15-mL tube. Place your tube upright in a test tube rack or beaker and let it stand undisturbed. After 5–10 minutes, you will begin to see the cells collect in the bottom of the tube. Maximum settling will occur after 20–30 minutes. Hold the tube up to the light to better see the cells sinking to the bottom of the tube. Some cheek cell samples will pellet more tightly and quickly than others and some will have a higher percentage of cells than others. This variation is a normal byproduct of the procedure.
6. Bring your 15-mL tube to the solution station. Using a plastic pipet, add 2 mL of cell lysis solution to your collected cheek cells. Use the graduations marked on the plastic pipet to measure the 2-mL amount.
7. Cap your 15-mL tube tightly and invert it 5 times. This action mixes the cell lysis solution with the collected cheek cell sample. Allow the tube to stand for 2 minutes.
8. Bring your 15-mL tube to the solution station. Hold the tube at an angle and, using a plastic pipet, carefully add cold 70% ethanol by running it down the inside of the tube. Add the ethanol until the total volume reaches 12–13 mL (use the lines on the side of the tube to help you measure). You should have two distinct layers. **Do not mix the cheek cell lysate layer with the ethanol layer.**
9. Watch closely as wispy strands of translucent DNA begin to clump together where the ethanol layer meets the cell lysate layer. The DNA will look like a cobweb extending up from the lysed cheek cell layer. Tiny bubbles in the ethanol layer will appear where the DNA precipitates.
10. Place your 15-mL tube upright in a test tube rack or beaker and let it stand undisturbed for a minimum of 10 minutes. During this time, DNA will continue to precipitate out of solution and extend like a ribbon through the entire ethanol layer. DNA yields will naturally vary within the class and not all DNA samples will extend through the entire ethanol layer.
11. Tie the ends of your embroidery string together with a knot to form a loop. Make sure the loop can fit over your head, as this will become your necklace string.
12. Use your plastic pipet to transfer your precipitated DNA out of the 15-mL tube and into the pendant tube (see Figure 1). Begin pipeting the DNA from the end of the most extended strand in the ethanol layer. As you pipet from this point, the DNA will be drawn up together. You should not move your pipet tip down into the cell lysate layer. If some of the DNA remains attached to the cell lysate layer, draw your pipet up until the DNA in your pipet detaches from that in the cell lysate layer. You do not need to transfer the entire precipitated DNA sample into your pendant tube. Before you expel your DNA into the pendant tube, allow it to sink to the tip of the pipet so that it will enter the pendant tube first. If the DNA does not sink, release ethanol into the 15-mL tube dropwise until the DNA is in the pipet tip. Expel the DNA into the pendant tube and fill the remaining space dropwise with ethanol. Do not overfill.

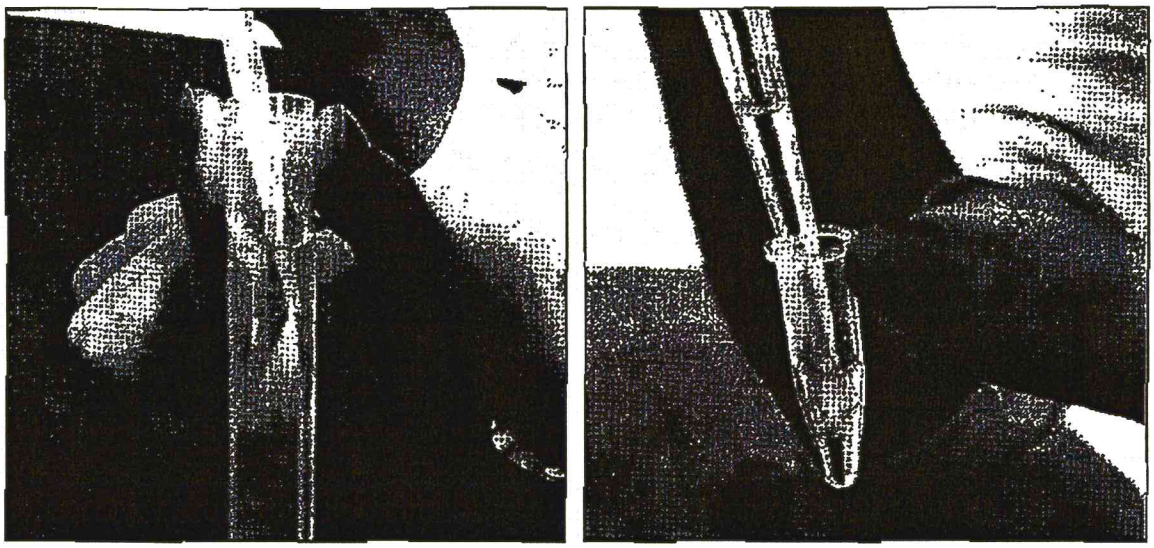

Figure 1. Transfer of precipitated DNA

13. With the pendant tube open, place the loop of your embroidery string around the cap hinge. Close the cap and put on your DNA necklace! Invert your pendant tube to see your DNA move through the ethanol.

Questions

1. Describe how long strands of double-helical DNA fit into the nucleus of a single cell.

2. Why is a sports drink used to collect the cheek cells instead of water?

3. What does the cell lysis solution do to the cells' membranes?