

Student Manual: Advanced Instruction

Cheek Cell DNA Extraction Capture Your Genetic Essence In a Bottle

Introduction

Deoxyribonucleic acid (DNA) is a molecule present in all living things, including bacteria, plants, and animals, and in almost all cell types. DNA is the carrier of genetic information and is responsible for determining a person's hair, skin, and eye color, facial features, complexion, height, blood type, and just about everything else that makes an individual unique. It also carries information required for cells to perform all of the functions that are common to all members of a species, or to all living things, and thus it is sometimes referred to as a biological "blueprint". Your personal blueprint is a combination of half of your mother's DNA (from her egg) and half of your father's DNA (from his sperm) during conception. All of your cells contain this complete set of instructions.

All DNA looks the same when it is extracted from cells, but it is exciting to look at your own DNA, knowing that this is really what makes you unique and alive. In this laboratory activity, you will extract your own DNA — a substance that holds your very own "blueprint" — from your cheek cells. You will use a quick and easy procedure that scientists routinely use to extract DNA from different organisms.

Every day scientists are making new discoveries as they study the information encoded in our DNA. Understanding DNA holds the possibility of curing diseases, the hope for millions who suffer from various genetic disorders and syndromes, making better products from biological sources, and even perhaps the key to longer life. We are beginning to understand who we are and why by studying our genetic material.

DNA Structure

At the molecular level, DNA looks like a twisted ladder or a spiral staircase. Two long molecules are aligned with each other, and the rungs are formed from pairs of chemical units called **bases**. This structure is referred to as a **double helix** because of the spiral, or helical form made by two strands. The bases function like letters in a code, so they are known as **A**, **G**, **T**, and **C** (abbreviations for their full names, adenine, guanine, thymine, and cytosine, respectively). **Each** base is connected to a sugar and a phosphate group, and the sugar and phosphate groups form the "backbones" of the ladder-like structure. (A **nucleotide** is one unit consisting of a base, sugar, and phosphate.) Scientists have found that **A** always pairs with **T**, and **G** always pairs with **C** in double-stranded DNA.

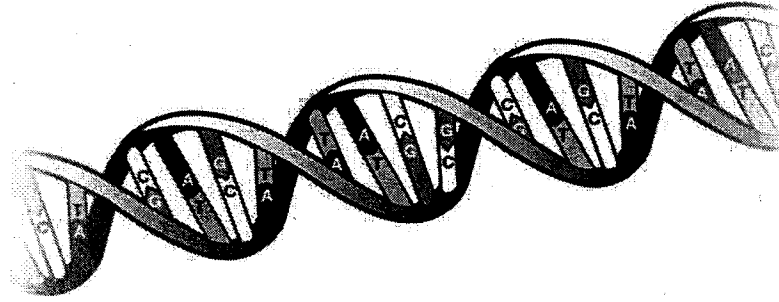


Fig. 3. A schematic representation of DNA (deoxyribonucleic acid). DNA is a long chainlike molecule that stores genetic information.

The 4 chemical letters of DNA are organized to make messages that can be understood by cells, called **genes**. These genes contain the information to make **proteins**, which are the basis for almost all of your body's structures and functions. Each of your cells contains several billion letters of DNA "text".

A DNA sequence is the particular arrangement or order of the bases along the DNA molecule. Human DNA sequences are 99.9% identical among each other. It is the <0.1% sequence variation that makes each of us unique. In other words, what makes you different from your classmate is an occasional difference in the sequence of bases in your genes.

The Genome, Chromosomes, Genes, DNA, RNA, and Proteins...What Is the Connection?

DNA is found within the nucleus of every cell in the human body, with the exception of mature red blood cells. The DNA is organized into structures called **chromosomes**, in which the long thin strands of DNA are tightly coiled around proteins. Every time a cell divides — for growth, repair, or reproduction — the chromosomes replicate in a highly organized process called mitosis. The 46 human chromosomes found in human cells are analogous to 46 volumes of an encyclopedia, which collectively contain all the information in your **genome**.

A **gene** is a section of DNA that contains the information to make a protein; it is like a written recipe that specifies the composition and order of assembly of a protein molecule. The human genome contains approximately 40,000 genes. The genome is analogous to a (gigantic) collection of cookbooks (remember, there are 46 "volumes" in the entire collection); not all of the recipes in a cookbook are prepared at once to make one meal, nor are all of the genes within the genome used in every cell. This selective gene expression according to cell type generates the characteristics of different cell types within your body. Basically, all of your cells contain the same books (chromosomes), but different cells read different recipes (genes) from the books.

Although genes specify the proteins that are made by cells, DNA is not the direct template for protein synthesis. The templates for protein synthesis are RNA (ribonucleic acid) molecules called messenger RNA (mRNA). Each mRNA molecule is simply a copy of the DNA sequence from one gene. mRNAs are the intermediates that carry the information from the DNA within the nucleus to the **ribosomes**, or protein manufacturers, within the cytoplasm. The ribosomes decode the genetic information and link together the appropriate amino acids to make the **protein** that is encoded by the gene. All the proteins made within a cell function to give the cell its traits.

Focus questions:

1. Imagine you are trying to explain the difference between chromosomes, genes, and DNA to your younger brother or sister who is two years younger than you. Write down your explanation in simple words that they could understand.
2. Does a liver cell contain the same chromosomes as a cheek cell?
3. If you wanted to isolate a copy of the gene that codes for a protein found in the stomach, could that gene be located in cheek cells? Explain your reasoning.

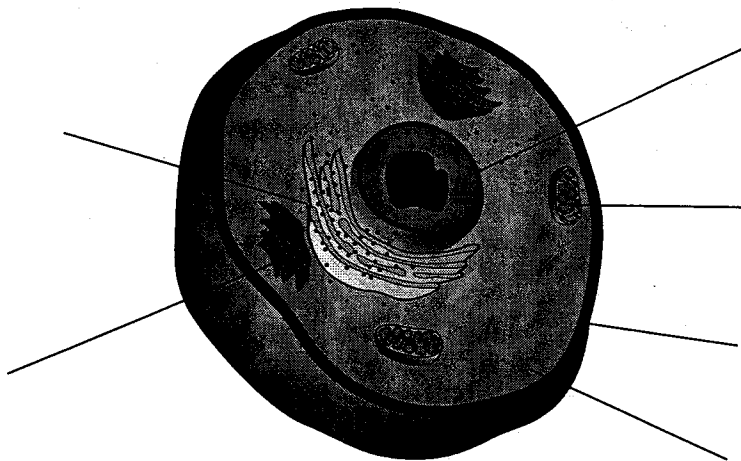
How can DNA be isolated from cells?

Step 1. Collecting epithelial cheek cells

The first step in DNA isolation is the collection of cells. The lining of the mouth is a good source of cells. These cells divide very often and are continually being sloughed off, making them an accessible source of cells. Simply by gently chewing your cheeks your cheeks and rinsing the inside of your mouth thoroughly with water allows you to collect a quantity of cells from which you can isolate your own DNA.

Focus questions:

Below is a schematic image of a cheek cell.



4. Label the cellular compartments, including the cell membrane, cytoplasm, and nucleus.
5. In which cellular compartment do you expect to find your genomic DNA?
6. Why is an intermediate like mRNA needed to copy the information from the genomic DNA so it can be translated into proteins?
7. What do you think will be the first step in isolating DNA from your cells?

Step 2. Lysing the cells and dissolving the phospholipid bilayer membranes

If you guessed that the first step of DNA extraction is to break open the cells, you are right! Detergent dissolves hydrophobic (oil-based) molecules, and the cell and nuclear membranes are mainly oil-based (you may have already heard of cell membranes being composed of "phospholipid bilayers"). After collecting cells from your cheeks, you will add a solution that contains detergent.

Focus questions:

8. Once the membranes have been dissolved, the DNA is released into the solution, but so are many other types of cellular molecules. List some types of molecules besides DNA that you would expect to find in a cell.
9. What method or agent do you think might be used to break down these unwanted molecules?

Step 3. Using protease to break down cellular proteins

As you may have already guessed, the most prevalent class of molecules that would interfere with the precipitation of pure DNA is protein. We can easily get rid of protein without damaging the DNA by using a specific enzyme that digests proteins, called a protease. Protease breaks the peptide bonds between the amino acids of proteins. By destroying all the proteins you will also eliminate DNases, enzymes that digest DNA (because enzymes are proteins).

Focus questions:

10. What proteins might be associated with DNA in the cell?
11. The protease used in this procedure functions best at 50°C. Would you expect this enzyme to be isolated from *E. coli* bacteria? Explain your answer. Hint: Where does *E. coli* live?
12. Meat tenderizer is often used to tenderize tough pieces of meat, like steak. Knowing that steak is made of protein-rich muscle tissue from cows, can you think of an explanation for how meat tenderizer works?

Step 4. Making DNA insoluble

The protease solution that you added to your sample also contains salt. The salt will cause the DNA to become less soluble in solution. DNA has a negative electrical charge due to the phosphate groups on the DNA backbone. When the salt is added, the positively charged sodium ions of the salt are attracted to the negative charges of the DNA, neutralizing the electrical charge of the DNA. This allows the DNA molecules to come together or aggregate instead of repelling each other.

Step 5. Precipitating the DNA with cold alcohol

To separate the DNA from the other molecules in the cell extract, you will add cold alcohol to your sample. Upon the addition of cold alcohol, the DNA will precipitate because it is less soluble in alcohol than in water. The colder the ethanol is, the less soluble the DNA will be in it. This is similar to the solubility of sugar in tea (or any drink); sugar dissolves more readily in hot tea than in iced tea.

In the presence of high salt and cold alcohol, the DNA that had been released from your cells precipitates and aggregates until it can be seen with the naked eye! The other molecules in the cell extract, such as the amino acids and carbohydrates, remain dissolved in the alcohol and water and will not be visible. It takes many thousands of strands of DNA to form a fiber large enough to be visible. Each strand will have thousands of genes on it, so you will be looking at material that contains millions of genes at once. Remember, though, that you are seeing the DNA from many thousands of cells all together.

Focus questions:

13. Match the outcomes on the left with the laboratory steps on the right.

- | | |
|------------------------------------|---|
| ___ Harvest the cells | A. Gently chew the insides of your mouth and then rinse vigorously with water |
| ___ Dissolve cell membranes | B. Add protease, incubate at 50°C |
| ___ Precipitate the DNA | C. Mix in a detergent solution |
| ___ Break down proteins | D. Layer cold alcohol over cell extract |
| ___ Make DNA less soluble in water | E. Add salt |

Cheek Cell DNA Extraction: Laboratory Instructions

Capture Your Genetic Essence In a Bottle

Workstation Checklist

Teacher's (Common) Station

Water bath at 50°C

Ice-cold bottle of 91% isopropanol or 95% ethanol on ice

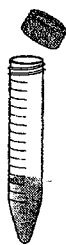
<u>Students' Workstation (4 students per station)</u>	<u>Number required</u>
15 ml tubes, each containing 3 ml water	4
Pink micro test tube labeled "prot", containing 1.25 ml protease + salt	1
15 ml tube labeled "lysis" containing 10 ml lysis buffer	1
Disposable plastic transfer pipets	6
Foam micro test tube holder	1
Permanent marker	1
Disposable paper cup or beaker for holding 15 ml tubes and subsequent waste collection	1

Procedure for DNA Extraction and Precipitation

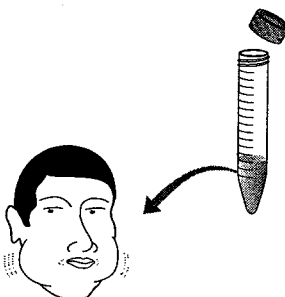
Steps 1 and 2: Collecting and breaking open cells

To collect as many cheek cells as possible, you will gently chew the insides of your mouth for 30 seconds and then rinse your mouth with a small amount of water. Ample cell collection is critical for success. For best results, make sure you spend the recommended amount of time collecting the cells.

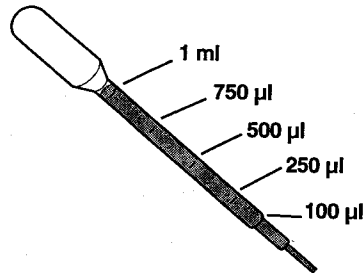
1. Obtain a 15 ml tube containing a 3 ml of water, and label it with your initials.



2. Gently chew the insides of your mouth for 30 seconds.
3. Take the 3 ml of water from your tube into your mouth and rinse vigorously for 30 seconds. Don't swallow the water!



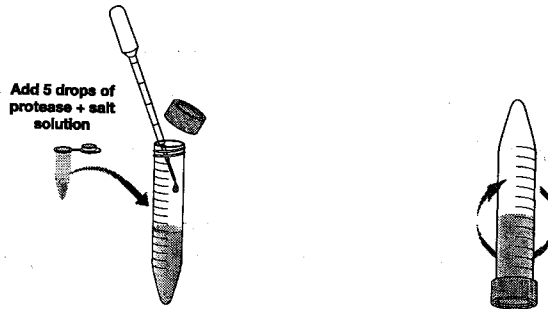
- Carefully expel all your water mouthwash back into your 15 ml tube.
- Locate the 15 ml tube at your workstation labeled 'lysis'. Using a fresh disposable plastic transfer pipet, add 2 ml of lysis buffer to your tube.



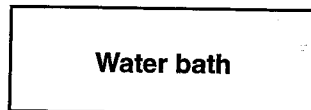
- Place the cap back in your tube. Gently invert your tube 5 times to lyse your cells. Don't shake the tube. If you observe any changes to your cells at this time, write them down.

Step 3: Removing proteins

- Obtain the pink tube labeled "prot" and add 5 drops of protease and salt solution to the 15 ml tube containing your cell extract. Cap the cell extract tube and gently invert it 5 times to mix.



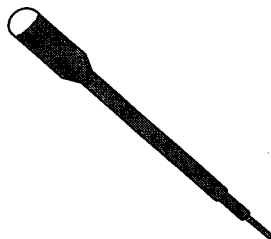
- Place your cell extract tube in the beaker or test tube holder in the 50°C water bath (at the common workstation) for 10 minutes to allow the protease to work.



50°C for 10 min

Steps 4 and 5: Making the DNA visible

- (You may need to do this step at the common workstation. Consult your teacher for specific instructions.) Fill a disposable transfer pipet with cold alcohol.



2. Tilt your 15 ml tube at a 45° angle and slowly add the alcohol, carefully letting it flow gently down the inside of the tube. Fill the tube with cold alcohol (about 10 ml total). You may need to use several pipets full of cold alcohol. You should be able to see two layers (upper and lower) forming. As you add the alcohol, pay close attention to the place where the alcohol and cell extract layers meet. Write down your observations.

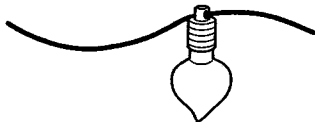


3. Place your 15 ml tube upright either on the cap or a test tube and leave it undisturbed at room temperature for 5 minutes.
4. After 5 minutes, look again at the contents of your tube, especially in the area where the alcohol and cell extract layers meet. Do you see anything? Write down your observations. Compare your sample with those of your classmates.
5. With the cap of your tube tightly sealed, mix the contents of your tube by slowly inverting the tube 5 times. Look for any stringy, white or clear material. **This is your DNA!**



6. If you are going to make a DNA necklace, your teacher will provide you with a glass vial. With a disposable plastic transfer pipet, carefully transfer the precipitated DNA along with approximately 750 μ l to 1 ml of the alcohol solution into the vial. Your teacher will help you seal the vial so you can complete the necklace.

If you are not going to make a DNA necklace, you can transfer and save your DNA in a fliptop micro test tube. With a disposable plastic transfer pipet, gently withdraw your precipitated DNA along with about 1 ml of alcohol solution and transfer it into the micro test tube. Tighten the cap and amaze your friends and family with your own DNA.



or

