Student Manual: Basic Instruction

Lesson 1

Cheek Cell DNA Extraction
Capture Your Genetic Essence in a Bottle

Introduction

What is DNA and what does it do?

Deoxyribonucleic acid (DNA) is a molecule present in all living things, including bacteria, plants, and animals. DNA carries genetic information that is inherited, or passed down from parents to offspring. It is responsible for determining a person's hair, eye, and skin color, facial features, complexion, height, blood type, and just about everything else that makes an individual unique. But it also contains all the information about your body that is the same in all human beings. In other words, your DNA is like a blueprint for your entire physical growth and development. Your DNA blueprint is a combination of half of your mother's and half of your father's DNA, which is why you have some features from each of your parents.

DNA contains four chemical units, referred to by the first letters in their names: **A** (adenine), **G** (guanine), **T** (thymine), and **C** (cytosine). These four DNA "letters" make up a code for genetic information. The letters of the DNA code are similar to the letters of our alphabet. The 26 letters in our English alphabet spell words, which can be arranged in infinite ways to create messages and information. Similarly, the 4 chemical letters of DNA are organized to make messages, called **genes**, that can be understood by cells. These genes contain the information to make **proteins**, which are responsible for almost all of your body's structures and functions. A gene is like a recipe, since it contains the all the information needed to make a protein.

Your DNA sequence is the particular arrangement or order of the chemical letters within your complete DNA collection, or **genome**. Scientists have determined that human DNA sequences are 99.9% identical. It is the <0.1% sequence variation from person to person that makes each of us unique. In other words, what makes you different from your classmate is an occasional difference in the letters of your genomes.

Where is DNA found?

The basic units of an organism's body are cells — they make up all of your tissues and organs (e.g., muscles, brain, digestive system, skin, glands, etc.). Cells are compartments with membranes made of protein and lipids (fats) that keep them separate from other cells. Within cells are further compartments with specialized functions. One compartment, called the **nucleus**, is like the cell's control headquarters and contains the DNA molecules, which are the master instructions for the functions of the cell. The DNA is organized into 46 tightly coiled structures called chromosomes. Every time a cell divides to make two identical new cells — for growth, repair, or reproduction — the chromosomes are copied, ensuring that the new cells will receive a full copy of the genetic blueprint for the organism.

What does DNA look like?

At the molecular level, DNA looks like a twisted ladder or a spiral staircase. The ladder actually contains two strands of DNA, with pairs of the chemical letters **A**, **G**, **T**, and **C** forming the rungs. This structure is called a DNA **double helix** because of the spiral, or helical form made by the two DNA strands. Each strand of DNA is very long and thin and is coiled very tightly to make it fit into the cell's nucleus. If all 46 human chromosomes from a cell were uncoiled and placed end to end, they would make a string of DNA that is 2 meters long and only 2 nanometers (2 billionths of a meter) wide!

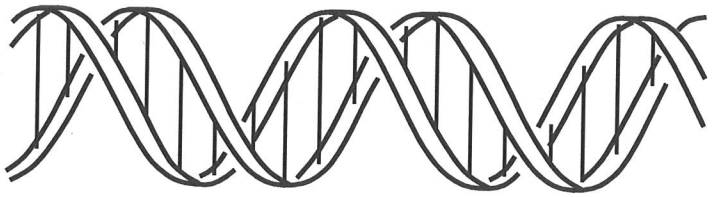


Fig. 2. A schematic representation of DNA (deoxyribonucleic acid). DNA is a long chainlike molecule that stores genetic information.

How can we make DNA visible?

Step 1: Collect cells

To see your DNA, you will collect epithelial cheek cells, break them open, and condense the DNA from all of the cells together. You can collect thousands of cells from the inside of your mouth just by gently chewing your cheeks and rinsing your mouth with water. The cells that line your mouth divide once or twice a day. Old cells fall off your cheeks continuously as new cells replace them. In fact, your cheek cells are coming off and being replaced every time you chew and eat food.

Focus question:

1. How could you test whether you were actually collecting cells from your cheeks? What piece of laboratory equipment might you use?

Step 2: Break open (lyse) the cells

Once you have collected your cells, the cells need to be broken open to release the DNA. Detergent will dissolve the membranes of your cells, just like dishwashing detergent dissolves fats and proteins from a greasy pan, because cell and nuclear membranes are composed of fats and proteins. Dissolving the membranes results in the release of the DNA. The process of breaking open the cells is called **lysis**, and the solution containing the detergent is called **lysis buffer**.

Focus questions:

2. When washing dishes, what works better, warm or cold water? Which do you think will help the detergent break open the cell, warm or cold temperatures?

3. Do you think your DNA will be visible after you have broken open your cells? Why or why not?

Step 3: Remove proteins

DNA is packaged tightly around proteins. Like spools for thread, these proteins keep the DNA tightly wound and organized so that it doesn't get tangled inside the nucleus. For you to see the DNA, it helps to remove the proteins so that the DNA can first loosen and expand, then collect into a mass with the DNA from all the other cells. You will incubate your lysed cheek cells with **protease**, which breaks down proteins so that they can no longer bind DNA. Protease is an **enzyme**, or protein machine, that works best at 50°C, which is the temperature of slightly hot water. The protease chews up the proteins associated with the DNA and also helps digest any remaining cell or nuclear membrane proteins.

Focus question:

4. Where do you think you would find proteases in your body? **Hint**: Where do the proteins that you eat get broken down?

Steps 4 and 5: Condense the DNA

Strands of DNA are so thin that it is not possible to see them when they are dissolved in solution. Think of the long, thin strands of DNA as fine white thread. If one long piece of thread were stretched across the room, it would be difficult to see. To make the thread more visible, you could collect it all together and pile it on the floor. In this laboratory experiment, you will use salt and cold alcohol to bring the DNA out of solution, or **precipitate** it. Salt and cold alcohol create a condition in which DNA doesn't stay in solution, so the DNA clumps together and becomes a solid mass that you can see.

Focus question:

5. Have you ever tried to add sugar to iced tea? Does the sugar dissolve easily? How does this compare to dissolving the same amount of sugar in the same amount of hot tea?

What does precipitated DNA look like?

Like salt or sugar, DNA is colorless when it is dissolved in liquid, but is white when it precipitates in enough quantity to see. As it precipitates, it appears as very fine white strands suspended in liquid. The strands are somewhat fragile — like very thin noodles, they can break if handled roughly. Also, if a mass of precipitated DNA is pulled out of its surrounding liquid, it will clump together, much like cooked noodles will clump together when they are pulled out of their liquid.

Cheek Cell DNA Extraction: Laboratory Instructions

Lesson 2

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

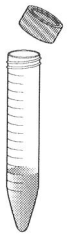
Students' Workstation (4 students per station)	Number required
15 ml tubes, each containing 3 ml water	4
Pink microcentrifuge tube labeled '**prot**', containing 1.25 ml of protease + salt	1
15 ml tube labeled '**lysis**' containing 10 ml lysis buffer	1
Disposable plastic transfer pipets	6
Permanent marker	1
Disposable paper cup or beaker for holding 15 ml tubes and subsequent waste collection	1
Microcentrifuge tube rack (optional for holding microcentrifuge tube with protease + salt solution)	1

Procedure for DNA Extraction and Precipitation

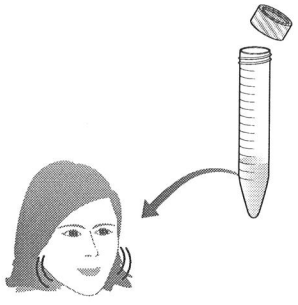
Steps 1 and 2: Collecting and Breaking Open Cells

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.

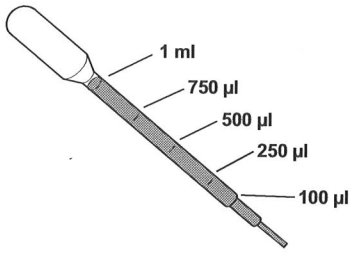
1. Obtain a 15 ml tube containing 3 ml of water, and label it with your initials.



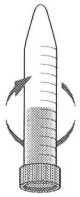
2. Gently chew the insides of your mouth for 30 seconds.
3. Take the 3 ml of water from your tube into your mouth and rinse vigorously for 30 seconds. Don't swallow the water!



4. Carefully expel all your water mouthwash back into your 15 ml tube.
5. 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.

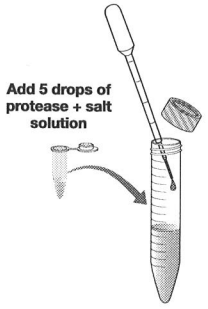


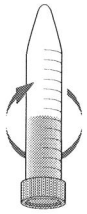
6. Place the cap back on your tube. Gently invert your tube 5 times to lyse your cells. Don't shake the tube. If you observe any changes to your cells at this time, write them down.



Step 3: Removing proteins

1. Obtain the pink microcentrifuge 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.





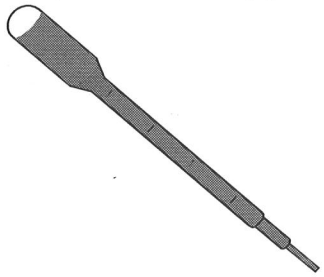
2. Place your cell extract tube in the beaker or test tube holder in the 50°C water bath (at the common workstation) for 10 minutes to allow the protease to work.

Water bath

50°C for 10 min

Steps 4 and 5: Making the DNA visible

1. (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. Obtain the tube of cold alcohol from your instructor or at the common workstation. Add 10 ml of the alcohol to your tube as follows. Hold your tube at a 45° angle and add the alcohol by slowly dispensing it down the inside wall of the tube. It will take repeated additions to add 10 mls. Screw the cap back onto your tube.

3. Place your 15 ml tube upright either in the cup or a test tube rack 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 helix vial, screw cap, and cord. With a disposable transfer pipet, carefully transfer the precipitated DNA along with approximately 500 µl of the alcohol solution into the vial, screw on the cap tightly.

   If you are not going to make a DNA necklace, you can transfer and save your DNA in a 1.5 ml microcentrifuge 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 microcentrifuge tube. Tighten the cap and amaze your friends and family with your own DNA!