Name _____
Date _____
Period ___

Cheek Cell 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.

The four 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 basses 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 species the composition and order of assemble 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, not 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 or your cells contain the same books (chromosomes), but different cells read different recipes (genes) from the books.

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

How can we make DNA visible?

Step 1: Collect cells

To see your DNA, you will collect cells, break them open, and condense the DNA from all of the cells together. You can collect thousands of cells form the inside of your mouth just by scraping it gently and thoroughly with a brush. The type of cells that line your mouth divides very often, coming off easily as new cells replace them continuously. In fact, these cells are coming off and being replaced every time you chew and eat food.

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**.

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.

Step 4 and 5: Condense the DNA (making DNA insoluble)

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 will cause the DNA to become less soluble in

the cell extract. DBA 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 to come together instead of repelling each other. 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. 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. The 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.

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.

Pre-lab 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 you explanation in simple words that they could understand.

_____

_____

_____

_____

2. Does a liver cell contain the same chromosomes as a cheek cell? Explain why or why not.

_____

_____

3. In which cellular compartment do you expect to find your genomic DNA?

_____

4. Why is an intermediate like mRNA needed to copy the information from the genomic DNA so it can be translated into proteins?

_____

_____

5. Once the membranes have been dissolved, the DNA is released into the solution, but so are <u>many</u> other types of cellular molecules. List some types of molecules besides DNA that you would expect to find in a cell.

_____

_____

6. What method or agent do you think might be used to break down these unwanted molecules?

_____

_____

7. What proteins might be associated with DNA in a cell?

_____

8. 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?

_____

_____

9. 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 tenderizer works?

_____

_____

_____

_____

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

1. ____ Harvest the cells	a. Scrape a brush against the inside of your cheek
2. ____ Dissolve cell membranes	b. Add protease, incubate at 50°C
3. ____ Precipitate the DNA	c. Mix in a detergent solution
4. ____ Break down proteins	d. Layer cold alcohol over cell extract
5. ____ Make DNA less soluble in water	e. Add salt

11. On a separate piece of paper, **make a flowchart** of the procedure for DNA extraction and precipitation. Next to each step **explain why the step was done**.

Quick Guide for DNA Extraction and Precipitation

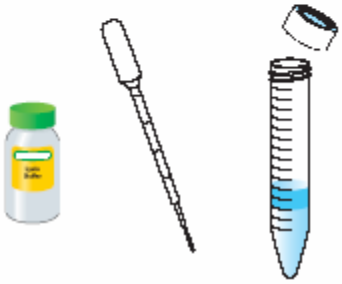
1. Obtain 15 ml tube containing 3 ml water from your instructor. Label the tube with your initials.

2. Gently chew the insides of your cheeks for 30 seconds. It is NOT helpful to draw blood!

3. Take the water from the 15 ml tube into your mouth, and swish the water around vigorously for 30 seconds.

4. Carefully expel the liquid back into the 15 ml tube.

5. Obtain the tube of lysis buffer from your workstation, and add 2 ml of lysis buffer to your tube.

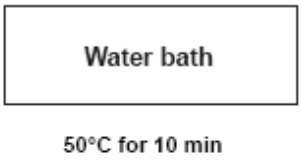


6. Place the cap on the tube, and gently invert the tube 5 times (don't shake your tube!). Observe your tube — do you notice any changes? If you do, write them down.

7. Obtain the tube of protease (**prot**) at your workstation. Add 5 drops of protease to your tube.

8. Place the cap on your tube, and gently invert it a few times.

9. Place your tube in a test tube rack or beaker in the water bath and incubate at 50°C for 10 minutes. Remove your tubes from the water bath.

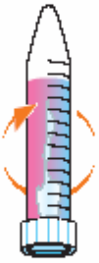


10. Obtain the tube of cold alcohol from your instructor or at the common workstation. Holding your tube at a 45° angle, fill your tube with cold alcohol, by adding approximately 10 mls to your tube. It will take repeated additions to add 10 ml of the cold alcohol using the disposable plastic transfer pipet.

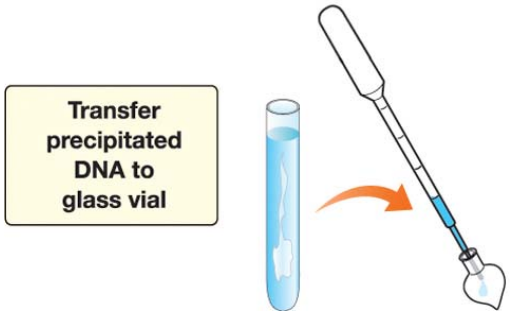
11. Place your cap on your tube, and let it sit undisturbed for 5 minutes. Write down anything you observe happening in the tube.

12. After 5 minutes, slowly invert the tube 5 times to help the DNA, which has begun to precipitate, to aggegate.

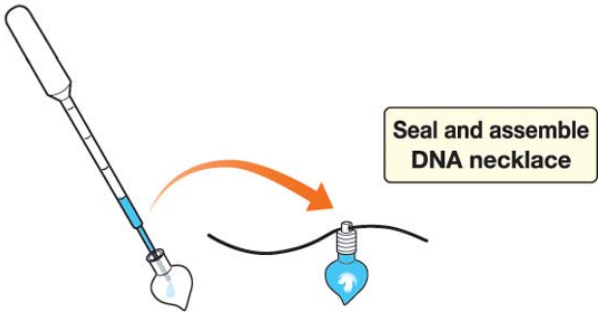
13. With a disposable plastic transfer pipet, carefully transfer the precipitated DNA along with approximately 750 µl to 1 ml of the alcohol solution into a small glass vial provided in the DNA necklace kit (166-2200EDU). If you are not going to make a DNA necklace, save your DNA in a flip-top tube provided in this kit.



14. Using a disposable plastic transfer pipet, carefully transfer the fluffy DNA strands you extracted into the small glass vial. Transfer as much of your DNA and as little alcohol as possible. The vial should be filled no higher than 2 mm from the top of the neck of the vial.



15. Firmly push the trimmed plastic stopper cap into the neck of the vial to seal the glass vial.
16. Slip the waxed cord through the silver cap.
17. Apply a small drop of super glue to the inside of the silver cap.



Post-Lab Questions:

1. Explain why each of the following materials were added to the cell extract:
   a. lysis buffer: _____
   _____
   b. salt solution: _____
   _____
   c. 95% ethanol: _____
   _____
2. Explain why each of the following conditions were applied to the cell extract:
   a. 50°C water bath: _____
   _____
   b. test tube tilted at a 45° angle prior to adding alcohol: _____
   _____
   c. cold ethanol: _____
   _____