Lab 8. Extracting DNA from Your Cells

Name _____

Lab Section ____ Team No. ____Date _____

Prelab Assignment

1. *Before* coming to lab, read all parts of this lab handout.
2. Answer the Prelab Questions 1 – 8 on pages 1 – 2 and be prepared to hand them in at the start of your lab class.

Goals of this Lab Exercise

After completing this lab exercise you should be able to.....

1. Extract DNA from some of your cells
2. Learn about the structure, function and replication of DNA

Prelab Questions

1. Why is DNA so important in biology? What is the function of DNA?

2. Where is DNA found in our bodies?

3. Draw a simple diagram of a cell, showing the cell membrane and the DNA in chromosomes surrounded by a nuclear membrane. *Label the following structures in your diagram*: Chromosome, DNA, nuclear membrane, plasma membrane

Extracting DNA from Your Cheek Cell. Cells from the lining of your mouth come loose easily, so you will be able to collect cells containing your DNA by swishing a liquid around in your mouth. The cells from the lining of your mouth also come off whenever you chew food.

4. How do you think your body replaces the cells that come off the lining of your mouth when you eat?

5. To extract DNA from your cells, you will need to separate the DNA (a nucleic acid) from the other types of biological molecules in your cells. What are the other three main types of large biological molecules in cells?

You will be using the same basic steps that biologists use when they extract DNA (e.g. to clone DNA or to make a DNA fingerprint). You will follow these 3 easy steps to extract the DNA:

Detergent
e**N**zymes (meat tenderizer)
Alcohol

<u>Read the procedure below and then answer the following three questions.</u>

6. What is the purpose of adding ***detergent*** when extracting the DNA from cells?

7. What is the purpose of adding ***enzymes*** (meat tenderizer) when extracting the DNA from cells?

8. What is the purpose of adding ***alcohol*** when extracting the DNA from cells?

Materials Needed to Extract DNA from Cheek Cells

Per lab table

- 1-Dropper bottle of liquid dish soap (0.25 mL per person or about 5 or 6 drops is needed per person)
- Small (12 × 75 mm) test tubes (tubes need to hold a minimum of 15 mL) (1 per student)
- 1-Test tube racks
- 1-Transfer pipettes

Per person

- Sports Drink like Gatorade (10 mL per student) in a 3 oz. Dixie cups
- String for necklace (2.5 ft per student)
- One 0.5-1.5 mL flip top microcentrifuge tube
- Glove (1 per student)

Per Class

- Meat Tenderizer (a pinch per student)
- 70-95% isopropyl or ethyl alcohol (ice cold—4 mL per student)
- Tub of ice, freezer, or refrigerator (1)
- Tub for dirty test tubes containing bleach (1% bleach solution to sterilize test tubes)

Procedure (Answer the numbered questions below as you perform each part of the procedure.)

Getting Your Sample of Cells (*Done by each person in the class!!*)

Obtain a cup with 10 mL of sports drink. You will need to get thousands of your cheek cells in the sports drink in order to extract enough DNA to see. Therefore you should *swish the sports drink around in your mouth vigorously for **at least one minute***. Then spit the drink back into the cup.

Step 1. Detergent

Add a small amount (*about 5 or 6 drops*) of liquid detergent to a test tube. Put a *glove on the hand you will use to hold your test tube*, not the hand you will use to pour. Now carefully pour the drink containing your cheek cells into the test tube with detergent until the tube is ***half full***.

- ***Why am I adding detergent?*** To get the DNA out of your cheek cells you need to break open both the cell membranes and the nuclear membranes. Cell membranes and nuclear membranes consist primarily of lipids. Dishwashing detergent, like all soaps, breaks up lipids. This is why you use detergents to remove fats (which are lipids) from dirty dishes. Adding the detergent to you cheek cell solution will break open the cell membranes and nuclear membranes and release your DNA into the solution.

Step 2. Enzymes

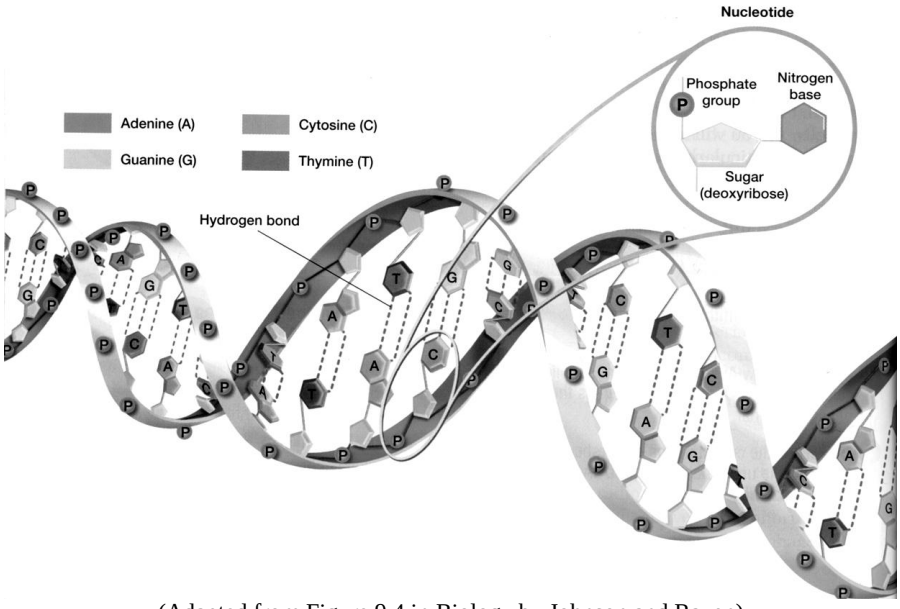
Add a pinch of enzyme (meat tenderizer) to your test tube. With your gloved thumb (or palm) covering the top of the test tube; gently invert the tube five times to mix. Place the tube in a test tube rack and let the mixture sit for *at least 10 minutes*. While you are waiting, you will learn about the structure of DNA. Remove your glove and throw it in the garbage.

- ***Why am I adding enzymes?*** The nucleus of each of your cells contains multiple long strands of DNA with all the instructions to make your entire body. If you stretched out the DNA found in one of your cells, it would be 2-3 meters long. To fit all of this DNA inside a tiny cell nucleus, the DNA is wrapped tightly around proteins. The enzyme in meat tenderizer is a ***protease***, which is an enzyme that cuts (digests) proteins into small pieces (i.e. into their constituent ***amino acids***). As this enzyme cuts up the proteins, the DNA will unwind and separate from the proteins.

9. The ***protease*** in meat tenderizer actually comes from plants, but animals also make proteases. Where in your body do you think you make protein-cutting (digesting) enzymes?

DNA Structure

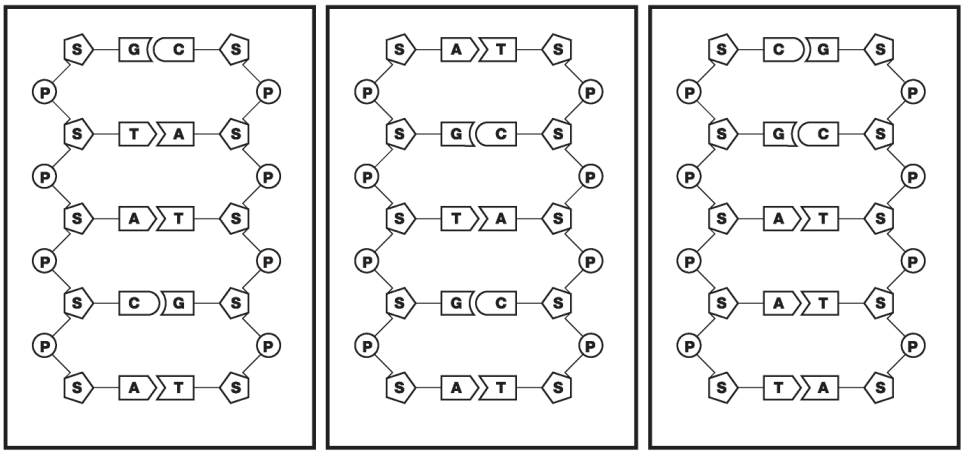
As you can see in the figure below, DNA consists of two strands of **nucleotides** wound together in a spiral called a **double helix**. Each nucleotide contains a phosphate and a sugar molecule called a **deoxyribose** (which explains why the complete name for DNA is deoxyribonucleic acid). Each nucleotide also has one of four different nitrogenous bases: **adenine** (**A**), **thymine** (**T**), **guanine** (**G**), and **cytosine** (**C**).



(Adapted from Figure 9.4 in Biology by Johnson and Raven)

The drawings below show a very small section of the DNA double helix from three very different organisms: a plant, a mammal, and a bacterium. Each strand of DNA shown contains five nucleotides, each with a:

S = five carbon sugar molecule called deoxyribose
P = phosphate group
A = adenine, **C** = cytosine, **G** = guanine, or **T** = thymine, the DNA nucleotide bases



Plant **Mammal** **Bacterium**

(From BioRad's "Forensic DNA fingerprinting kit" http://www.bio-rad.com/cmc_upload/Literature/12525/4006096G.pdf)

You can see that the phosphate from one nucleotide is bonded to the sugar in the next nucleotide to form the backbone of each strand in the DNA molecule. The bases of the nucleotides in each strand of DNA extend toward each other in the center of the DNA double helix molecule. A crucial aspect of DNA structure is the **base-pairing rule**: **A** in one strand always pairs with **T** in the other strand, and **G** in one strand always pairs with **C** in the other strand. You will see later that this base-pairing is crucial for the cell to make new copies of each DNA molecule in preparation for cell division.

10. Compare the sugar-phosphate arrangement in the backbone of the DNA from the plant, the mammal and the bacterium. Are there any differences?

11. a.) Which bases are present in the DNA of the plant? The mammal? The bacterium?

    b.) Are the bases present in all three cases in the same order? _____

12. a.) Describe the pattern of base pair matching for the two strands in the plant's DNA. In other words, which types of bases are paired together?

    b.) Does the DNA from the mammal follow the same base-pairing rule as the DNA from the plant?_____

    c.) Is base-pairing the *same* or *different* in the DNA of the bacterium? (*Circle your choice.)*

13. What is the only characteristic that differs between these segments of DNA from a plant, a mammal and a bacterium?

14. *Complete the following statement*. These observations illustrate the similarity of the basic structure of DNA in all living organisms. The genetic differences between plants, mammals and bacteria are due to differences in the _____ of bases in their DNA.

Step 3: Alcohol

Using a pipette, *slowly* add cold rubbing alcohol into the test tube; let the alcohol run down the side of the test tube so it forms a layer on top of the soapy liquid. Add alcohol until you have ***about 2 cm*** of alcohol in the tube. Alcohol is less dense than water, so it floats on top. Place the tube in the test tube rack and **do not mix or bump the test tube for 10 minutes.** DNA molecules will clump together where the soapy water below meets the cold alcohol above, and you will be able to see these clumps of DNA as white strands. While you are waiting for the DNA to become visible you will learn about DNA replication. *Save the pipette* for extracting the DNA from the test tube when making the "necklace" as described on page 7.

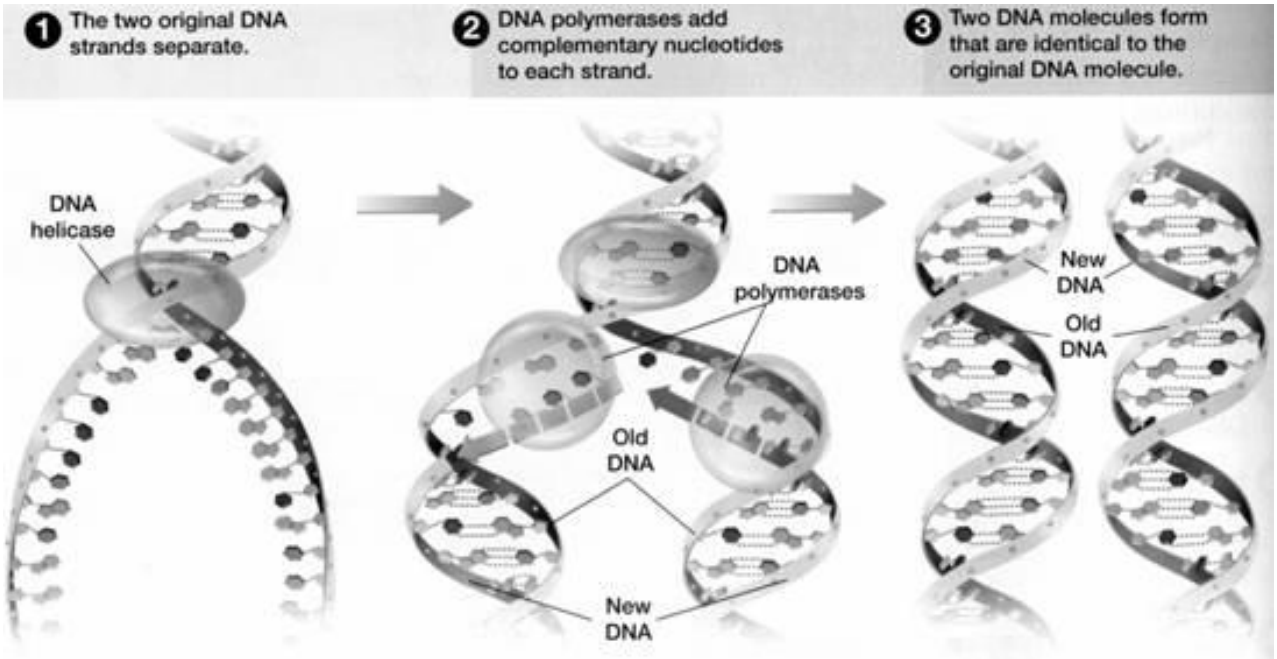
- ***Why am I adding alcohol?*** The cold alcohol reduces the solubility of DNA. When cold alcohol is poured on top of the solution, the DNA precipitates out into the alcohol layer, while the lipids and proteins stay in the solution.

DNA Replication

Cells in our body are dividing all the time. For example, cell division in the lining of your mouth provides the replacements for the cells that come off whenever you chew food. Before a cell can divide, the cell must make a copy of all the DNA in each chromosome; this process is called ***DNA replication***.

15. Why is DNA replication necessary before each cell division?

As shown in the figure below, the first step in DNA replication is the separation of the two strands of the DNA ***double helix*** by the enzyme ***DNA helicase***. After the two strands are separated, another enzyme, ***DNA polymerase***, forms a new matching DNA strand for each of the old DNA strands. DNA polymerase forms the new matching DNA strand by adding nucleotides one at a time and joining each new nucleotide to the previous nucleotide in the growing DNA strand. Each nucleotide added to the new strand of DNA follows the base-pairing rule with the matching nucleotide on the old strand of DNA. The result is two identical DNA double helixes.



(Adapted from Figure 9.9 in Biology by Johnson and Raven)

16. In the drawing below, the small segment of ***plant DNA*** (from page 4) is shown after the two strands of the DNA molecule have been separated by ***DNA helicase***. Your job is to play the role of ***DNA polymerase*** and create the new matching strands of DNA to make two pieces of double-stranded DNA in the drawing below—don't forget to add the sugar-phosphate "backbone" of each new strand you make! Use the base-pairing rule to determine which nucleotides to add.

17. a.) Now look at both of the double-stranded pieces of DNA you have created. Are there any differences between the two strands? ***Yes*** or ***No*** *(Circle your choice.)*

    b.) Are these new double-stranded pieces of DNA the ***same as*** or ***different than*** the original piece of plant DNA (shown on page 4)? *(Circle your choice.)*

During actual DNA replication sometimes mistakes are made and the wrong nucleotide is added to the new strand of DNA. ***DNA polymerase*** can "***proofread***" each new double helix DNA strand for mistakes and backtrack to fix any mistakes it finds. To fix a mistake it finds, DNA polymerase removes the incorrectly paired nucleotide and replaces it with the correct one. If a mistake is made and not found, the mistake can become permanent. Then, any daughter cells will have this same change in the DNA molecule. These changes are called ***point mutations*** because they change the genetic code at one point, i.e. one nucleotide. Point mutations can result in significant effects, such as the genetic disease, sickle cell anemia.

Making Your Necklace

By now your DNA should be visible as clumps of white strands floating in the alcohol layer. There may be air bubbles attached to the strands.

Use a *pipette* to suck up your DNA from the test tube and transfer it to the small capped tube. Be careful to squeeze the air out of the pipette before you put the pipette in the test tube; then gently suck up your DNA. Fill the small capped tube the rest of the way with alcohol. Close the cap of the tube around a piece of string. Now you have a necklace with your very own DNA!

Questions

18. Which of the following do you think will contain DNA? *Circle your choices and explain your reasoning*.

    a.) bananas b.) concrete c.) fossilized insect in amber d.) meat e.) metal f.) spinach g.) apple

19. Describe the function of ***helicase***.

20. Describe the *two* major functions of ***DNA polymerase***.

21. Explain why each part of the name DNA polymerase (DNA, polymer, -ase) makes sense.

22. What is a mutation? Why do mutations occasionally occur?

23. Explain why most mutations are harmful, but occasionally they might be beneficial to an organism.

Acknowledgement: This activity is adapted from the Genetic Science Learning Center's "How to Extract DNA from Any Living Thing" (http://learn.genetics.utah.edu/units/activities/extraction/) and BioRad's "Genes in a bottle" (http://www.biorad.com/cmc_upload/Literature/54133/4110034B.pdf), by Jennifer Doherty and Dr. Ingrid Waldron, Department of Biology, University of Pennsylvania.