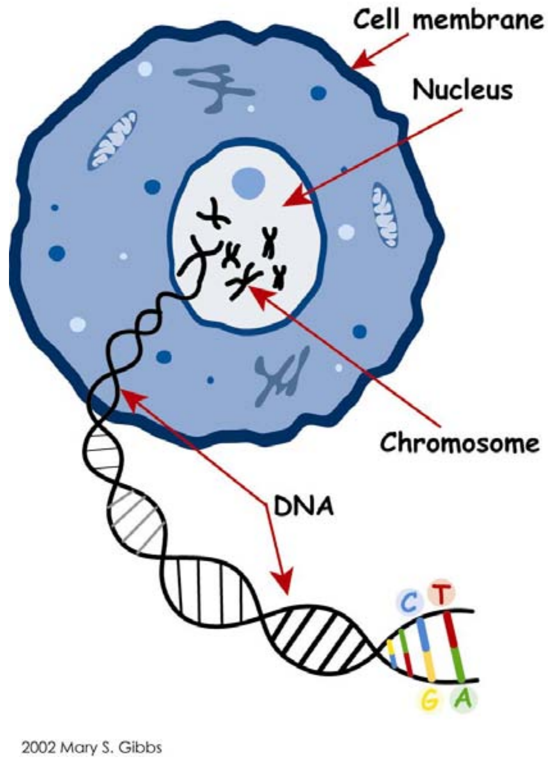
This DNA extraction laboratory is an activity to facilitate learning about cells and the structures inside of them. All living things are made of cells. Inside cells are smaller structures called organelles that work to perform different functions, or jobs, within the cell. Included are a pre-laboratory activity to aid in understanding organelle functions and a laboratory activity in which students will isolate and observe DNA from two types of cells.

Background Information



All living things are made of cells. Cells are the basic unit of life and make up all plants, animals and bacteria. In plants and animals, cells often work together to form tissues; groups of these tissues are called organs. For example, heart cells make up heart tissue, which in turn makes up the organ called the heart. The cells in your heart work together to push red blood cells through your body. Red blood cells carry oxygen to all parts of your body and oxygen is used to produce energy so your body can survive.

Inside cells are smaller structures called organelles. These tiny structures act like factories that help the cell perform certain tasks such as general repairs, removing waste, and reproduction.

The three main parts of the cell are the **nucleus**, which holds **DNA**, the **cell membrane**, which surrounds and protects the cell, and the **cytoplasm**, which is the jelly-like part of the cell between the membrane and the nucleus. All of the smaller organelles, such as **mitochondria**, are found in the cytoplasm.

Cell Part	Function in the Cell
Cell Membrane	Security guard: checks what goes in and out of the cell
Mitochondria	Powerhouses: generates energy for the cell
Membrane Receptors	Gather information and deliver it to the nucleus
Nucleus	The “brain” or control center for the cell
DNA	Contains instructions for the cell, found in the nucleus

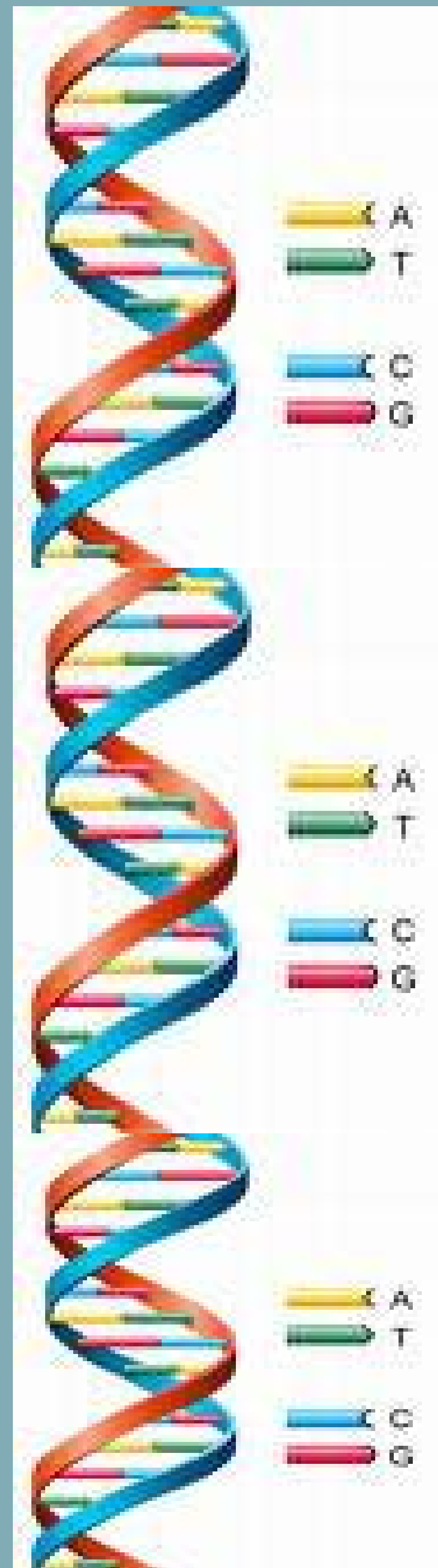
Deoxyribonucleic acid or **DNA** is the molecule that controls everything that happens in the cell. DNA contains the genetic code or commands that direct the activities of cells and ultimately, the body. DNA is present in all living things from bacteria to animals. In animals, it is found in almost all cell types except for usually in red blood cells.

DNA is made of two spiral strands that wind around each other like a twisted ladder. The rungs of the ladder are made up of nucleotides: **adenine (A)**, **thymine (T)**, **cytosine (C)**, and **guanine (G)**. These nucleotides pair together: adenine with thymine and cytosine with guanine. These A-T and C-G pairs make up the rungs of the ladder. The different nucleotides are like a four-letter alphabet and can spell out different words or codes. A **gene** is a long series of the four letters (nucleotides) that contains instructions for the cell to make a particular protein.

DNA is the largest known molecule. A single unbroken strand can contain millions of atoms. When DNA is released from a cell it typically breaks up into tiny fragments. These tiny fragments have a slightly negative electric charge. Salt ions, common in many solutions, are attracted to the negative charges on the DNA fragments and prevent them from adhering to one another. By controlling the salt concentration of the solution containing the DNA fragments, DNA can remain fragmented or become very "sticky" and form large globs of molecular material.

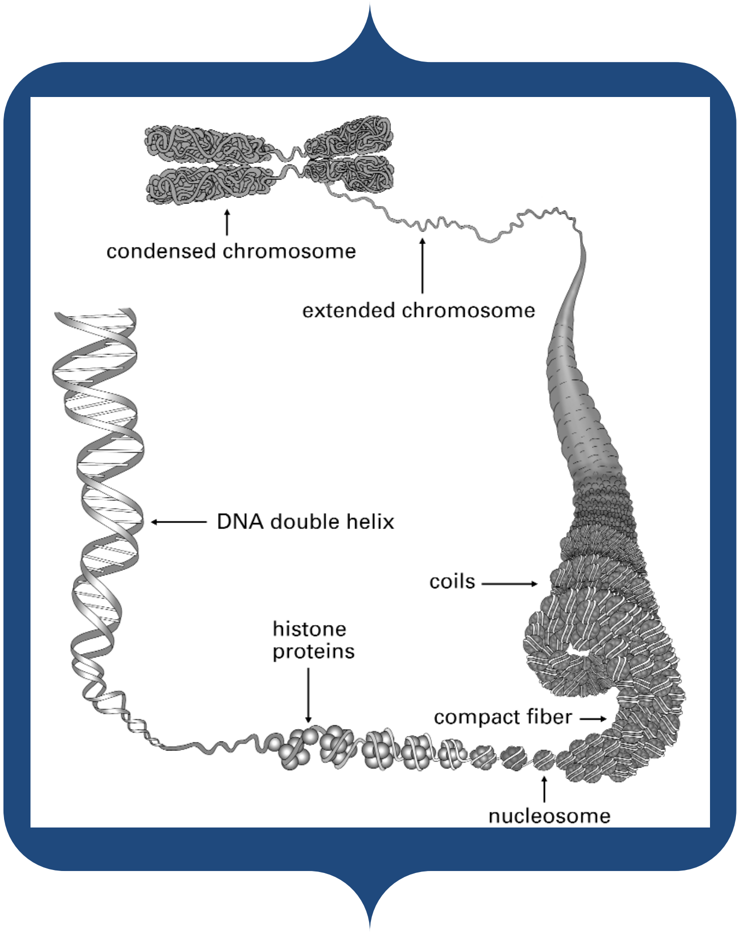
Since DNA is an essential molecule to all living things (with the exception of some viruses), it is not surprising that elaborate mechanisms to protect it have evolved. In order to extract DNA successfully, it is helpful to understand these protective mechanisms.

The simplest organisms do not have the protection of a nucleus; this group of organisms is called **prokaryotes**. Common prokaryotes are bacteria. The DNA of bacteria floats around in the cytoplasm and is protected from invading viral DNA by **restriction enzymes** that can cut foreign DNA into small pieces. So how do the bacteria prevent their own DNA from being digested by these enzymes? The bacterial DNA has chemical groups called **methyl groups** attached to it which prevents the restriction enzymes from cutting it. Bacteria have a relatively simple protection mechanism. As organisms get more complex, so does the protection of their own DNA.

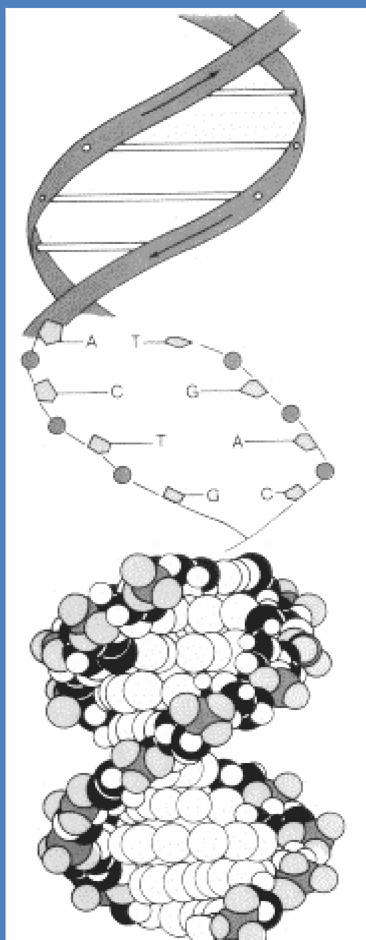


Eukaryotic organisms keep their DNA contained within a nucleus, protecting it from activities going on in the cytoplasm. Plants have the extra protection of a cell wall. All eukaryotes have DNAse enzymes floating around in their cytoplasm that can cut DNA. In order to extract spoolable DNA, it is necessary to denature, or breakdown, these enzymes before rupturing the nucleus. Heat or pH changes are often used to denature proteins and enzymes. DNA is a relatively sturdy molecule but it is very long and can break when it is removed from the nucleus. If the DNA is broken or sheared in too many places, it won't spool and is harder to capture effectively. It is important to be relatively gentle in the last steps of DNA extraction and to avoid violent shaking or mixing that would shear the DNA.

The process of isolating DNA requires that it be released from a cell whether it is a plant (which has extra protection with a cell wall), animal, fungi, or bacterium. Detergents and soaps breakdown cell membranes and proteins so that the DNA can be released. Protein enzymes or **proteases**, like those in contact lens cleaner or "Ultra" forms of laundry detergent, can be used to further this process of breaking down proteins.



Once the DNA fragments are released into solution, the DNA can be spooled together by using ice-cold alcohol. A small layer of alcohol is added to the top of the solution containing the cellular fragments. The DNA will collect at the interface between the alcohol and the cell solution. The DNA can then be captured, or spooled, onto a wooden stick or glass rod. The alcohol allows the DNA fragments to stick together once again and you have a blob of DNA to examine. Although this method is effective at isolating DNA, the DNA is by no means pure. Other materials like protein and cell fragments are carried along. Additional steps can be completed to remove proteins and cellular debris, thereby purifying the isolated DNA.

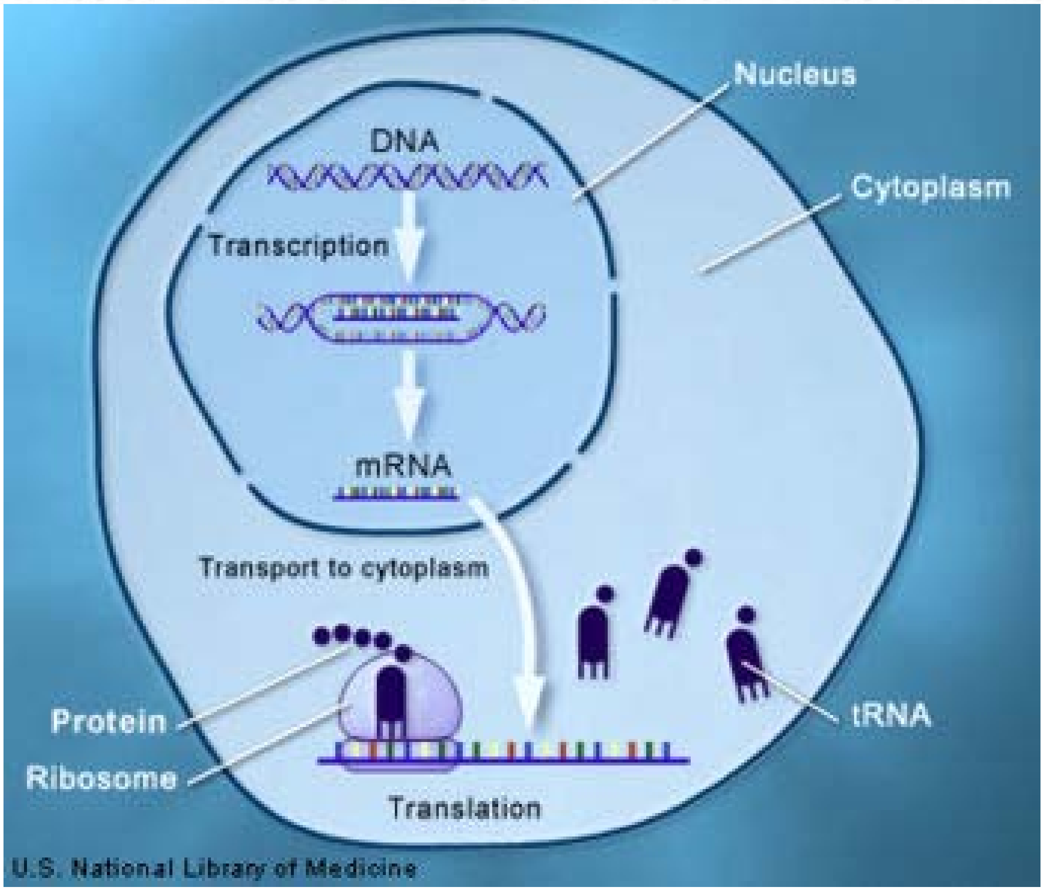


The structure of DNA.
DNA is composed of a series of nucleotides which bind to each other through hydrogen bonding. In DNA, adenine always pairs with thymine and cytosine always pairs with guanine. The joining of the two DNA strands by hydrogen bonding forms the characteristic double helix structure of DNA.

Photo source: http://academy.d20.co.edu/kadets/lundberg/images/biology/dna71.gif

DNA

Every cell in the human body, with the exception of gametes and mature red blood cells, contains the same complete set of genetic information. Humans have about three billion base pairs of DNA in their genome, but only about three million bases, or **about 0.1% of the genome, vary between individuals**. This means that for any two random individuals, about 99.9% of their genetic sequence is exactly the same. The exceptions to this are related individuals, who are more genetically similar, and identical twins, whom theoretically have exactly the same genetic code (given no mutations). Despite this striking level of similarity, individuals will have unique sequences within this 0.1% variation that can identify them from any other individual in the world. Since the development of DNA profiling, several methods for identification have been employed. Each method begins with the collection of DNA. DNA is commonly collected from sources such as blood, saliva, and hair follicles, but almost any cell type can be used.



DNA REPLICATION

MAKES MULTIPLE COPIES OF COMPLEMENTARY DNA

TRANSCRIPTION

CREATES RNA FROM THE DNA FOUND IN THE NUCLEUS

TRANSLATION

RNA MADE IN THE NUCLEUS LEAVES THE NUCLEUS THROUGH A NUCLEAR PORE AND ENTERS THE CYTOPLASM

THERE ARE THREE TYPES OF RNA MADE:

mRNA = MESSENGER RNA

tRNA = TRANSFER RNA

rRNA = RIBOSOMAL RNA

USING A PIPETTE AIDE

1. Examine the pipette:
    - Numbers ⇧ on one side = for drawing up fluid
    - Numbers ⇩ on the other side = for liquid expelled from the pipette.
2. Insert the pipette into the chunk portion of the pump with slight pressure.
3. Gently twist counter clockwise; this assures a secure fit.
4. Hold the pipette close to its upper end.
5. Place second and third finger on the top and bottom buttons.
    - The button on top is used to expel liquid out of the pipette.
    - The button on the bottom is used to draw liquid into the pipette.
6. To loosen the pipette:
    - Hold it near the chuck.
    - Twist slightly clockwise and pull the pipette out from the chuck portion of the Pipette Aide.

Micropipette Explanation

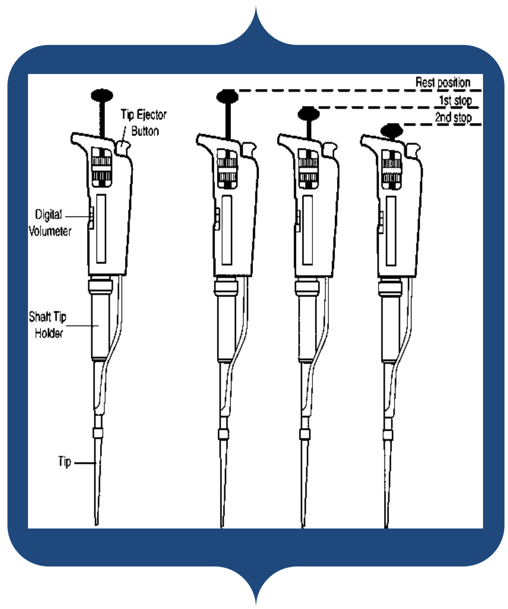
1 L = 1,000 mL **1 mL = 1,000 µL**

Micropipette Procedure

1. Adjust the pipette to the desired volume by turning the dial. Do not turn the dial beyond the volume range for the pipette.

2. Firmly press a new tip onto the pipette by inserting the pipette into the tip while the tip is still in the box.

3. Get tip out without touching it with your hands; this is to prevent contamination of the samples.



4. Draw up liquid from the micropipette
   - Depress the plunger to the first stop to measure the desired volume and hold the plunger in that position.
   - Holding the pipette vertically, immerse only the very end of the tip into the liquid to be transferred.
   - Slowly release the plunger to draw up the liquid.
   - Wait 1 – 2 seconds to be sure the full volume of sample is drawn up into the tip.

5. Dispense the liquid
   - Place the tip into the container where the liquid is to be released.
   - Slowly depress the plunger to the second stop to blow out all of the liquid in the tip.
   - Remove tip out of liquid.
   - Release plunger carefully.
   - Eject tip into a waste container.

Lab associates,

This past week I have made an amazing discovery – inside of different cells I have found a material I have never seen before! Unlike the proteins that we have found in cells, this new substance comes from the nucleus of the cell and is not damaged by protease enzymes. Thus, it cannot possibly be anything we already know of. I have been calling it nuclein.

It is very interesting to try different cells and materials to see what contains nuclein. Through my experiments, I have found nuclein in both cells from a cow and from salmon, but have not been able to find any in water.

I am very interested to see if nuclein is also in plants and would like for you to set-up an experiment using a fruit or vegetable.

Good luck!

Johann Meischer

DNA Extraction PART I:

1. Identify the samples you will use in your experiment today:

Positive Control: ____________________________
This is the sample expected to contain DNA.

Negative Control: ____________________________
This is the sample not expected contain DNA.

Experimental S: ____________________________
You will determine if the sample contains DNA.

Experimental K: ____________________________
You will determine if the sample contains DNA.

Experimental B: ____________________________
You will determine if the sample contains DNA.

2. Write a hypothesis about your experimental sample, using an "if/then" statement. Make sure your hypothesis includes:
   - whether the experimental sample will or will not contain DNA
   - a comparison to the (positive or negative) control you believe the experimental samples will resemble

Experimental Sample S (Strawberry):

____

____

Experimental Sample K (Kiwi):

____

____

Experimental Sample B (Banana):

____

____

3. Be sure you have five capped test tubes:
   - (-) for negative control which contains 2 mL of distilled water
   - (+) positive control tube which contains 2 mL of salmon cells
   - (B) for experimental sample banana
   - (K) for the experimental sample kiwi
   - (S) for experimental sample strawberry
   - Be sure you have eight labeled plastic pipettes:
     S, K, B, dH_2O, DNA Buffer, 3% SDS Buffer, NaCl, and Ethanol.

4. Take the experimental Samples:
   - **S (strawberry)**
   - **K (kiwi)**
   - **B (banana)**

5. Using the plastic pipettes labeled **dH_2O**, add 7 mL of distilled water (dH_2O) to each zip-lock bag:
   - **S (strawberry)**
   - **K (kiwi)**
   - **B (banana)**

6. **Seal the bag.**

7. Using the plastic pipettes labeled **DNA Buffer**, add 3 mL of DNA buffer to each zip-lock bag:
   - **S (strawberry)**
   - **K (kiwi)**
   - **B (banana)**

8. **Remove the air from the zip-lock bag.**

9. **Seal the bag.**

10. Gently mash up the experimental sample within the zip lock bag:
    - **S (strawberry)**
    - **K (kiwi)**
    - **B (banana)**

11. Identify the beaker labeled **S** with the cheesecloth over the top of the beaker.

12. Carefully open the bag and pour off all of the liquid experimental mixture (avoid solid chunks) into the beaker.

13. Squeeze the cheesecloth to make sure all liquids are within the beaker.

14. Identify the beaker labeled **K** with the cheesecloth over the top of the beaker.

15. Carefully open the bag and pour off all of the liquid experimental mixture (avoid solid chunks) into the beaker.

16. Squeeze the cheesecloth to make sure all liquids are within the beaker.

17. Identify the beaker labeled **B** with the cheesecloth over the top of the beaker.

18. Carefully open the bag and pour off all of the liquid experimental mixture (avoid solid chunks) into the beaker.

19. Squeeze the cheesecloth to make sure all liquids are within the beaker.

20. Use the plastic pipette labeled **dH_2O** to add 2 mL of distilled water into the tube labeled **–** which represents the negative control.

21. Examine the **+ (positive control)** test tube in the test tube rack.

22. Make sure there is 2 mL of liquid solution in the test tube labeled **+.**

23. Use the plastic pipette labeled **S** to add 2 mL of the liquid experimental sample into the tube labeled **S**.

24. Use the plastic pipette labeled **K** to add 2 mL of the liquid experimental sample into the tube labeled **K**.

25. Use the plastic pipette labeled **B** to add 2 mL of the liquid experimental sample into the tube labeled **B**.

26. **Add 1 mL of DNA buffer** (using the **DNA Buffer** pipette) to each of the white capped test tubes.

    Be careful not to touch the tip of the pipette to the inside of the test tubes or your samples may become contaminated.

27. Re-cap the tubes and mix well by inverting.

28. Allow the tubes to sit in the rack for at least **5 minutes** (the longer you wait, the better your results).

29. **Add 2 mL of ethanol** (using the **Ethanol** pipette) slowly down the side of each of the white capped tubes to form a layer that floats on top of each sample. **DO NOT MIX THE SAMPLE**.

    If DNA is present it should precipitate out in white or clear clumps that may also look like cobwebs or gooey strands.

30. **Which samples contained DNA? Circle your answers:**

Negative Control:	YES	NO
Positive Control:	YES	NO
Experimental S:	YES	NO
Experimental K:	YES	NO
Experimental B:	YES	NO

Did you notice white particles in your sample? YES NO

What are the white particles floating around in your sample?

Were the hypotheses supported by the data you observed? Explain.

Experimental S (strawberry):

Experimental K (Kiwi):

Experimental B (banana):

Instructions for Extracting Cheek Cells

1. Using the first swab, roll along the inside of one of your cheeks for 30 seconds. Holding the handle between your thumb and forefinger, roll the swab along the inside of one of your cheeks. As you roll the bristles, apply moderate pressure along with a brisk up and down motion. Imagine you are using a roller brush to paint a surface and you'll have the technique down! (Brush the cheeks for 30 seconds)

2. Carefully place the brush into the lysis buffer vial, rotating vigorously in the solution, then run all sides of the brush along the rim of the vial numerous times until you see that all or most of the fluid has been removed from the swab. It is imperative that you do this step carefully and thoroughly so as to make sure we get as many cells as possible. Rotate brush in a circular as well as up and down motion. (Be careful not to splash the fluid out – we don't want to waste your cells!) Run the brush along vial rim in a downward motion numerous times. Make sure to rotate the brush so as to get all sides.

3. Scrape bristles along the edge in a downward motion. Releasing as many cells as possible into the lysis buffer vial.

4. Dispose of the used swab.

5. Using the 2nd swab, repeat steps 1 through 4 this time on the other cheek.

PART II: Extracting Cheek Cells

1. Identify the 15 mL conical tube containing Lysis buffer at your station.
2. Identify the micropipette at your station that measures up to 1000 µL.
3. Make sure the micropipette is set to **750 µL.**
4. Measure **750 µL of 3% SDS Lysis Buffer** into the microcentrifuge tube.
5. Take a new sterile cheek swab out of its package.
6. **REMEMBER TO KEEP THE CHEEK SWAB STERILE!!!**
7. Roll this swab along the inside of your left cheek for 30 seconds = 30 wipes.
8. Place the swab into the 3% SDS Lysis Buffer contained within the microcentrifuge tube.
9. Swirl it around 10 times.
10. Scrape the swab along the inside of the vial. The goal of this step is to remove as many cheek cells from the swab as possible.
11. Throw the swab away.
12. **DO NOT USE THIS SWAB AGAIN!!!!**
13. Take a new sterile cheek swab out of its package.
14. **REMEMBER TO KEEP THE CHEEK SWAB STERILE!!!**
15. Roll the swab along the inside of your right cheek for 30 seconds = 30 wipes.
16. Again, swirl the swab around in the microcentrifuge tube containing 3% SDS Lysis Buffer.
17. Scrape the swab along the inside of the microcentrifuge tube. The goal of this step is to remove as many cheek cells from the swab as possible.
18. Throw the swab away.
19. Be careful not to spill the fluid**.** **You don't want to lose your cells!!!**

PART III – Extracting DNA from your Cheek Cells

1. Once the cheek cells have been placed into the 750 µL of 3% SDS Lysis Buffer:
    - Cap the microcentrifuge tube.
    - Invert the tube 10 times; so the cells and buffer are completely mixed.
2. Identify the micropipette at your station that measures up to 200 µL.
3. Set the micropipette to **150 µL**.
4. Add **150 µL of 5 M NaCl** (salt solution) to the collected DNA sample. The DNA and salt will turn cloudy and white. This precipitate, the cloudy white substance, is the suspended DNA from your cheek cells.
5. Place the cap carefully on top of the microcentrifuge tube.
6. Invert the microcentrifuge tube 10 times.
7. Place the microcentrifuge tube on the table at your station.
8. Identify the micropipette at your station that is set to 750 µL.
9. Take the 15 mL conical tubes labeled **"ETHANOL."**
10. In one hand hold the Pipette Aide with the tube tilted at an angle.
11. **Add 750 µL of "ICE COLD ETHANOL"** to the small microcentrifuge tube **very slowly!**
12. The DNA will begin to precipitate fully within 4-5 minutes and will float up to the top of the ethanol layer.
13. Transfer the DNA (at the top) into the amulet using a transfer pipette.
14. Seal the amulet tube with the silicone stopper.
15. Have the instructor cut off the excess stopper and glue the silver cap on.
16. When this has dried, run the cord through and wear your DNA necklace!

How to seal our glass charms using our plastic stoppers



Step 1: Place stopper securely in vial. Do not force it in but gently apply pressure in a circular motion until a good seal is made. These stoppers may not go in all the way. They are made with larger tops for leverage and a wider base to make a good seal. Example: using a cork to seal a bottle.



Step 2: Simply cut off the excess using a razor. This will leave a flat clean surface to place some glue on and make your cap go on with ease. The glue will also seal off the top making it more air tight. (You can also elect to just trim off the excess so your cap will fit. Finger nail clippers does an excellent job if you choose to do this.)



Step 3: Place some glue in your cap and over the sealed top of the vial and them place together. You can wipe any excess glue that comes out. Let it dry accordingly. You're done!

STUDENT DATA/OBSERVATIONS

Part II – Extracting Your Cheek Cells

1. Describe what you saw when you put your cheek cells in the lysis buffer.

____

____

Part III – Extracting Your DNA

2. Describe what you saw on the top of your microcentrifuge tube when you mixed your cells with the ethanol.

____

____

3. Describe what you saw on the bottom of your microcentrifuge tube when you mixed your cells with the ethanol.

____

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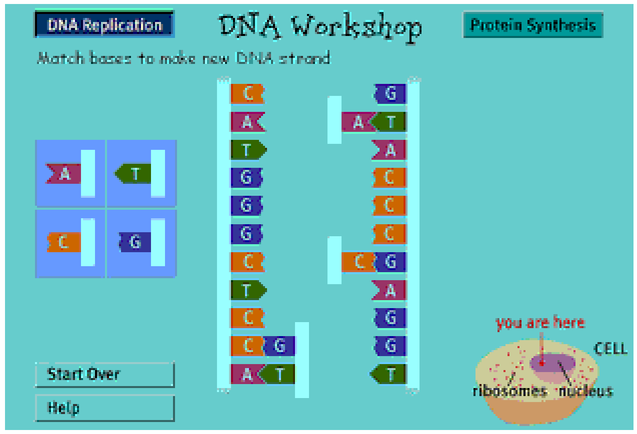
4. Describe what the DNA looks like before you put it in the amulet.

____

____

DNA Replication



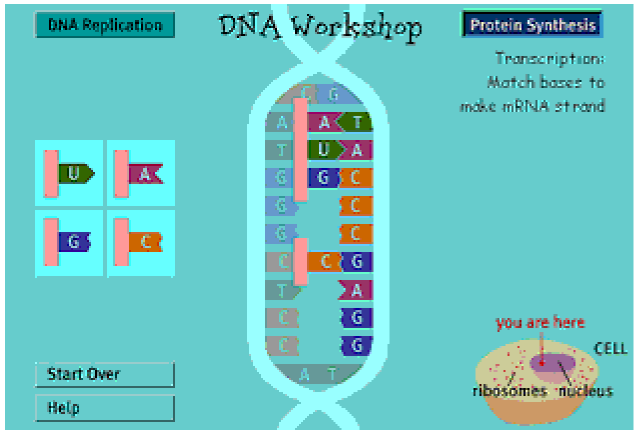
Below is a list of nucleotides and their corresponding base pairs:

A = Adenine pairs with T = Thymine
G = Guanine pairs with C = Cytosine
C = Cytosine pairs with G = Guanine
T = Thymine pairs with A = Adenine

Match the DNA nucleotide bases to the templates shown below.

DNA OLD		DNA NEW	DNA NEW		DNA OLD
A	-			-	**T**
T	-			-	**A**
C	-			-	**G**
C	-			-	**G**
T	-			-	**A**
G	-			-	**C**
A	-			-	**T**
A	-			-	**T**
C	-			-	**G**
C	-			-	**G**
T	-			-	**A**

Transcription



DNA has Thymine and RNA has Uracil.

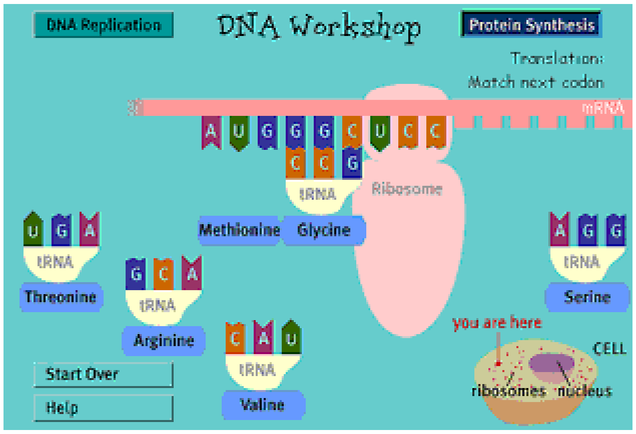
When RNA is created from DNA, the Adenine in DNA is paired with Uracil in RNA.

Match bases of mRNA to the nucleotides being carried on the tRNA molecules.

A = Adenine pairs with U = Uracil
G = Guanine pairs with C = Cytosine
C = Cytosine pairs with G = Guanine
T = Thymine pairs with A = Adenine

DNA	**mRNA**
A -	
T -	
C -	
C -	
T -	
G -	
A -	
A -	
C -	
C -	
T -	

Translation = Protein Synthesis



Match the tRNA anticodon (group of three nucleotides found on tRNA) to the mRNA codon (group of three nucleotides found on mRNA).

mRNA: AUG GGC UCC CGU UCC ACU GUA

tRNA: ____

Identify the amino acid chain made as a result of this process:

Methionine---Glycine--- ________________---___________________---_________________---

________________---________________ . . .