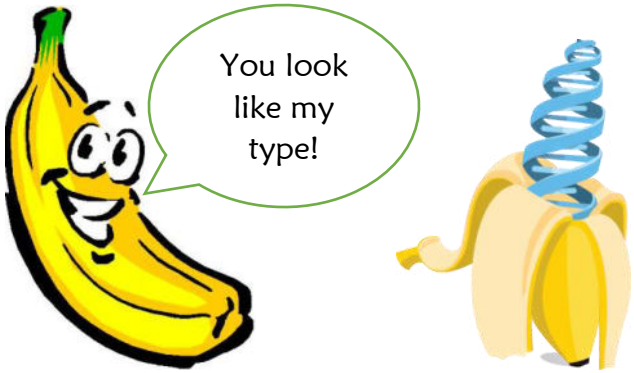
Name: _____ Period: _____

Banana DNA Extraction Lab



Objectives:
Describe where DNA is located in a plant cell. Explain what procedures are required to release DNA from a plant cell Observe the extraction of Genomic DNA from plant cells.

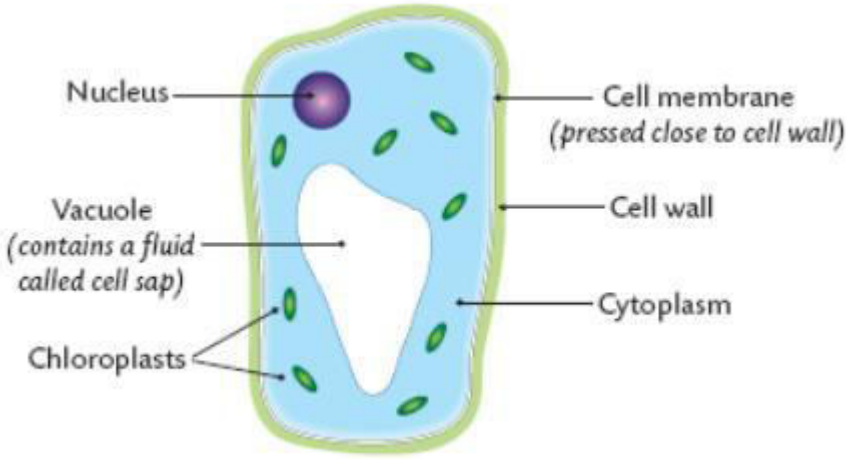
Why go bananas?
What can we tell about the molecular structure of DNA by studying the characteristics of DNA when we purify it from bananas?

Background: We will break open the cells of bananas and isolate the DNA from the rest of the cell debris. Bananas are a good source of DNA because some bananas are diploid (2 copies of each chromosome – 22 chromosomes in total) and others are even triploid banana (3 copies of each chromosome). You will never be able to eat a banana again without thinking of how much DNA is in it!

Introduction:
The process of isolating DNA from a cell is the first step of many laboratory procedures in biotechnology. The scientist must be able to separate the DNA from the unwanted substances of the cell gently enough so that the DNA is not broken up and destroyed. A "filtrate" is made of bananas and treated with a ***buffer*** (buffers prevent wide swings in pH) containing salt (NaCl) and distilled water. The salt solution acts as a buffer to maintain a constant pH and binds up the positive ions to prevent enzymes (DNAses) from chewing up the DNA. The salt shields the negative phosphates of the DNA which allows these ends to come closer so that they can ***precipitate*** (recall precipitates are solids that result from chemical reactions) out of a cold alcohol solution. A detergent is added which causes the cell membrane to break down by ***emulsifying*** the lipids (emulsifying allows water and fat to play nice together!) and proteins which allows the cell membrane to break apart.

PRELAB:

How do we isolate DNA? Take a look at the sketch of the plant cell below. The chromosomes (which are made of DNA) are in the nucleus.



List in order all of the structures of the plant cell that we have to go through in order to get to the DNA in the nucleus.

_____

_____

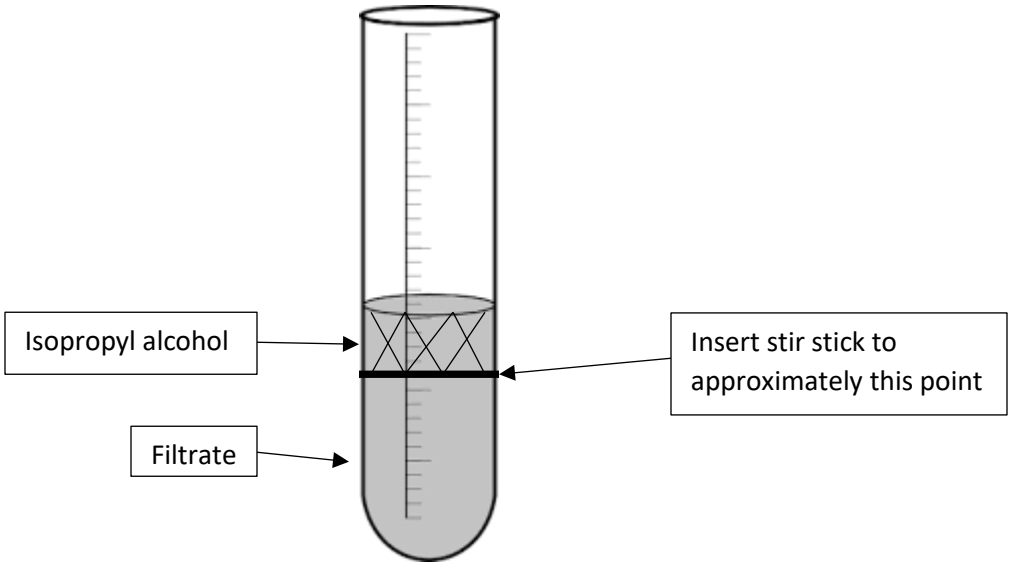
Materials:

- Ice-cold 95% ethanol or 95% isopropyl alcohol
- Prepared, buffered banana "shake"
- Large Test tube
- Coffee filter
- Wooden stir sticks
- 200 ml beaker
- Rubber bands (small)

Procedure

1. Place the coffee filter you have been provided with over the lip of the beaker and fold so that it's held in place. Secure with a rubber band.
2. Ask your teacher to pour around 50 mL of the buffered banana "shake" into the filter. After 10 minutes you should have some clear liquid at the bottom of your beaker. This liquid is called ***filtrate***. Gently stir the remaining liquid in the filter (careful is the word here, you don't want to tear the filter!). Wait another two minutes, then remove the filter and place it in the trash (**hint: carry the beaker over to the trash and then remove the filter**).
3. Using a pipette, transfer 3 full pipettes of the clear liquid (or all you collected if you don't have that much) into the provided test tube.

4. Have your teacher dispense some chilled isopropyl alcohol over the filtrate in your test tube. Return the test tube to the test tube rack and wait four minutes (do not disturb the solution – DO NOT STIR OR SHAKE!!) The white material you may see coming out of the filtrate and into the isopropyl alcohol is the banana's DNA.
   Carefully inset the wooden stir stick near the point where the alcohol meets the filtrate (See the illustration below). Gently twirl the stir stick. As more strands emerge from the filtrate they will start to collect (spool).



Factoids:

- The banana and you share about 50% of the same DNA.
- The DNA in your cells comes in threads of chromatin. If these threads were placed end-to-end they would stretch to about 2 meters in length.

Questions:

1. Describe the appearance of the DNA you extracted.

2. What was the purpose of blending the bananas to the point they were liquefied?

3. What was the purpose of the filter in our lab?

4. What happened when the isopropyl alcohol was added to the filtrate?

5. Is DNA found in all living or once living cells?

6. Since the bananas were once living, and we extracted DNA from them, what does this mean about the foods you eat? Does cooking them affect the DNA in any way?

7. Do you think your results would be different if you were to use a different fruit or vegetable? **Explain**.

Teacher Preparation

Buffer:

1L distilled water
15 g NaCl
15 mL clear hand soap (containing EDTA)
15 g baking soda

Mix the above ingredients and place into a flask or beaker. Chill in advance. Note: the ingredients will dissolve more effectively in warm water, just make the day ahead and chill as directed.

Banana slurry ("shake")

1 large banana
500 mL of the chilled buffer solution
1 – Blender or smoothie maker

Place the banana in the blender. Add the buffer solution and liquefy. Place in a cooler or fridge.

Also, place your bottle of isopropyl in a cooler or fridge until well chilled.

I find that a little mini-cooler with ice chills better than a mini-fridge. And the colder you can get the isopropyl the better.