ACTIVITY: EXTRACTING DNA (DNA = deoxyribonucleic acid) FROM COMMON FOOD INGREDIENTS

DNA Extraction – Onion method #1:

Ingredients:
Isopropyl alcohol*
Ice/water bath
½ onion
distilled water
¼ tsp sodium chloride (table salt) (1.5 g) 1 mL
¾ tsp sodium hydrogen carbonate (baking soda) (5 g) 4 mL
1 tsp name brand dish soap or liquid detergent 5 mL
*traditional experiments recommend 99%, but this is not readily available; 70% is readily available from most pharmacies

Materials:
1 paring knife
1 cutting board
Ice bucket or container for ice solution
Measuring cups or 125 mL and 250 mL Erlenmeyer flasks
Strainer or funnel
Paper towels or small coffee filters
Glass stirring rods or wooden stir sticks
Clear bowls or test tubes

1. Place isopropyl alcohol in ice/water bath or in the freezer to cool thoroughly.

2. Buffer solution:
   125 mL of water (distilled water, if available) ½ cup
   1 mL (1.5 grams) of sodium chloride (table salt) ¼ tsp
   4 mL (5.0 grams) baking soda (sodium hydrogen carbonate) ¾ tsp
   5 mL of good quality liquid laundry detergent, 1 tsp

Make the buffer solution by pouring placing all the ingredients into a clean 250 mL Erlenmeyer flask or clean container. Chill the buffer solution by placing the flask in a larger beaker filled with crushed ice and water.

Note: *buffer solutions are used in this lab for several reasons.*

- *the saltiness and acidity (pH) of the solution is very close to that in living things; as a result, the DNA will like to dissolve into this solution.*
- *the detergent is added to help break down cell walls in the onion cells. Cell walls in living things are made of long polar molecules with a "greasy" end and a charged end. Because detergent is used to break apart greasy particles from dishes or clothes, it will also work to tear apart the "greasy" molecules in cell walls. It is important that the cell walls break down in this lab, so that the DNA inside the cell can be seen.*

Note: *chilling the buffer solution is important because DNA are still extremely fragile, and break apart easily when removed from cells. To slow down the rate at which the DNA breaks up, it is important to cool down the buffer solution to near freezing. Because there is less energy available to encourage a reaction to take place, there is less chance for the DNA to break up if the buffer solution is chilled.*

3. Dice ½ an onion coarsely. Use a mortar and pestle to mash the pieces of onion into a pulpy sludge; or, place the diced pieces of onion into a beaker or 125 mL flask and mash them with the blunt end of a test tube. Careful! Don't use too much force or the test tube and/or beaker will break!

Note: *mashing the onion allows the cell walls to break, releasing the DNA into the juice, or at the very least expose the cell walls so the detergent can break them down.*

4. Place 10 mL (2 tsp) of the onion mush/juice into a small, clean beaker or flask and mix in 20 mL (4 tsp) of the chilled buffer solution. Stir vigorously with a stirring rod for 3 minutes.

Note: *by the exposing the onion to the detergent solution, the detergent will break up the cell walls, releasing the onion DNA into the buffer solution.*

5. Pour as much liquid as possible into a clean test tube. Let the test tube sit in a crushed ice/water bath for 5 minutes. In this time, the solids should settle to the bottom of the test tube, and the top should mainly be liquid.

6. Fit a small coffee filter or filter paper into a strainer/funnel and place over another clean test tube. Ensuring that the pulp is left behind, pour the remainder of the solution through the filter until the test tube is about half full.

Note: *The solution in the test tube, should be clear. It consists of dissolved DNA fragments, as well as some other biochemical compounds such as RNA and some proteins. DNA is a very long molecule, but compared to the holes in a piece of filtering paper, the molecule is still small enough to pass through.*

7. Pour the ice-cold isopropyl alcohol gently along the side of the test tube, until there's about 1 ½ inch 4 cm) topping the DNA solution. The goal is to have the alcohol stay on the top of the DNA solution, with as little mixing as possible.

Note: *Generally, molecules are attracted to the boundaries of two liquids – and sometimes the concentration of large molecules can be much higher at the boundary between two liquids. If the DNA is attracted to the surface, most of it will be able to be pulled out. However, if the alcohol and onion juice mix too much, there will be too much alcohol throughout the whole liquid, and the DNA won't be attracted to the surface, making it much harder to pull any out of the tube.*

8. <u>Very gently</u> insert a coffee stirrer or glass rod through the upper alcohol layer in the test tube into the DNA containing buffer solution. While disturbing the solution as little as possible, leave the glass rod or stirrer in one place and rotate it in 1 direction; if successful the DNA fragments will wind onto the stick in the same way that thread winds onto a spool.

Note: *DNA spools onto the stick or glass rod because the exposed ends have polar chemical groups on them. Glass and wood are also polar, so the ends of the DNA are attracted to the stirrer. By winding the stirrer, you are basically just reeling in the DNA molecules.*

9. After twirling the stick for about 60 seconds, pull the stirrer up through the alcohol layer. The DNA should be adhered to the end of the stick and appears as a transparent, viscous sludge at its tip. The molecule that has collected on this stick consists of the entire genetic code for the making of an onion.

Note: *When the DNA is pulled through the non-polar alcohol layer, it clumps together because it would rather be attached to polar materials such as the stick or even itself. Remember, "like dissolves like", meaning that polar compounds will tend to want to stay in polar environments while non-polar compounds will want to stay in non-polar environments. In this case, DNA, a polar compound, sticks to itself simply because it prefers a polar environment (itself) to a non-polar environment (the rubbing alcohol).*

10. Clean up: The waste left over from this lab should consist of a soapy onion paste, a soapy onion liquid, some isopropyl solution, and a large amount of onion goo. All solutions can be poured down the sink, while the onion goo should be wrapped in a paper towel and garbaged. The collected DNA is a totally safe compound, and can be thrown away or saved to show others.

DNA Extraction – Onion method #2:

Ingredients:
2 onions
Distilled water
Isopropyl alcohol*
¼ cup name brand dish soap or liquid detergent 50 mL
*traditional experiments recommend 99%, but this is not readily available; 70% is readily available from most pharmacies

Materials:
1 paring knife
1 cutting board
Blender
Measuring cup or 250 mL Erlenmeyer flask
Strainer
Paper towels or small coffee filters
Clear plastic forks or glass stirring rods
Clear bowls or test tubes

Procedure or Protocol:

1. Peel and cut onions in eights, and place onions in blender. Add 125 mL (½ cup) water; adding extra 15 mL (1Tbsp) if required for good blending. Blend coarsely – about 5 seconds. <u>If you puree the onion too finely, the filtering step will be very slow.</u>

2. Pour into small container or beaker. Add 50 mL (¼ cup) liquid soap. Stir gently to prevent creating a foamy mess. Let soap incubate with onions for 5 minutes.

3. Line strainer with paper towel or coffee filter and place strainer over beaker. Pour the onion/ soap/ water solution into the lined strainer. Strain liquid from onion mixture. This should take about 10-15 minutes.

4. Divide liquid contents into 5 clear plastic cups or test tubes. Gently, drizzling along the sides of the container, pour in an equal volume of isopropyl alcohol.

5. Examine the interface between soap solution and isopropyl alcohol, and look for some white stringy stuff -DNA! Allowing light to shine through the container will make it easier to see the DNA. Allow the mixture to sit for a few minutes, and an increasing amount of DNA will come out of solution.

6. After 5 minutes or so, mix gently with fork or stirring rod -long strands of DNA should spool onto rod.

DNA Extraction #3 – Yeast Method:

Ingredients:
Dry yeast
5 g Adolph's meat tenderizer
Isopropyl alcohol*
Ice/water bath
275 mL distilled water 1 cup + 2 Tbsp
30 mL non-iodized salt 2 Tbsp
45 mL hot tap water 3 Tbsp
20 mL name brand dish soap or liquid detergent 4 tsp
*traditional experiments recommend 99%, but this is not readily available; 70% is readily available from most pharmacies

Materials:
Ice bucket or container for ice solution
Measuring cups or 125 mL and 250 mL Erlenmeyer flasks
Blender
Glass stirring rods or wooden stir sticks

1. Place isopropyl alcohol in ice/water bath or in the freezer to cool thoroughly.
2. Mix 1 package of dry yeast with 45 mL (3 Tbsp) of 50^{o}C (122^{o}F) hot tap water. Mix until yeast is dissolved. Keep mixture covered and warm for about 20 minutes.
3. Make a buffer solution by mixing 20 mL (4 tsp) detergent, 20 g non-iodized salt, and 175 mL (3/4 cup) distilled water.
4. Make a 5% tenderizer solution by combining 5 g of Adolphe's meat tenderizer with 95 mL (6 Tbsp + 1 tsp) distilled water.
5. After 20 minutes, add 45 mL (3 Tbsp) of the buffer solution to the yeast.
6. Place mixture in a blender and blend on high for 30 seconds to 1 minute.

7. Pour yeast-buffer mixture back into the beaker. Add 15 mL (1 Tbsp) meat tenderizer solution. Stir to mix.
8. Place 5 mL (1 tsp) of the mixture into a test tube.
9. Slowly, pour 5 mL (1 tsp) of the cold isopropyl alcohol carefully down the side of the tube, allowing the alcohol to form a layer above the DNA solution. The goal is to have the alcohol stay on the top of the DNA solution, with as little mixing as possible.
10. Let the mixture sit undisturbed for 2-3 minutes until bubbling stops.
11. Very gently insert a coffee stirrer or glass rod through the upper alcohol layer in the test tube into the DNA containing buffer solution. While disturbing the solution as little as possible, leave the glass rod or stirrer in one place and rotate it in 1 direction; if successful the DNA fragments will wind onto the stick in the same way that thread winds onto a spool.
12. After twirling the stick for about 60 seconds, pull the stirrer up through the alcohol layer. The DNA should be adhered to the end of the stick and appears as a transparent, viscous sludge at its tip. The molecule that has collected on this stick consists of the entire genetic code for the making of yeast.