Extraction of DNA from an Onion

DNA was first discovered in the 1950’s, but there is still a lot to know about it, especially how it is used to determine physical traits that we all have, and how it regulates the workings of the body. It is important for us to remember that DNA is just a chemical [deoxyribonucleic acid]. Because it is a chemical we can do reactions with it just like we can work with any other chemical. In his lab, we will use the chemical properties of DNA to extract it from the cells of onions.

**Materials:**

* Buffer solution [made from 120 mL water, 1.5 g sodium chloride, 5.0 g sodium bicarbonate and 5 mL shampoo]

What is the buffer for?

1. The saltiness and acidity [pH] of the solution is very close to that in living things, as a result, the DNA will be like to dissolve in this solution.
2. The shampoo [or 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 shampoo is used to break apart greasy particles in your hair, it will also work to tear apart the “greasy” molecules in cell walls.
   * Cell walls must break in order for us to get inside the cell where the DNA is.

* Coffee stirrer
* Coffee filter
* Rubbing alcohol [ice cold]
* Stirring rod
* Crushed ice/water bath in 400 mL beaker
* Onion
* Mortar and pestle
* Straw
* Test tube [2]
* Graduated cylinder
* 50 mL beaker
* Funnel

**Procedure:**

1. Gather all materials. Except the buffer solution
   * + The buffer solution must be chilled for the following reasons.
       - * *As important as DNA molecules are to life, they are still extremely fragile, and break apart easily when removed from cells. To slow down the rate, at which DNA breaks up, we cool down the buffer solution to nearly freezing. Chemical reactions always take place slower in cold solutions than in warm ones, because there is a lot less energy around to make the reaction to take place.*

2. Mash a piece of onion into a pulpy sludge.

* *The onion must be mashed so that we can either break the cell walls [releasing the DNA into the juice] or at the very least expose the cell walls so the shampoo can break them down.*

3. Place 10 mL of onion mush/juice into a small, clean beaker or graduated cylinder and mix in 20 mL of the chilled buffer solution. Stir vigourously with a stirring rod for 3 minutes

* *In this step, we’re exposing the onion to the shampoo solution. The shampoo will break up the cell walls, releasing the onion DNA into the buffer solution.*

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

5. Making sure to leave the pulp behind, strain the rest of the solution through a coffee filter with a funnel into another clean test tube. The test tube should be about half full when you have finished; if you have more pour some out.

* *The [hopefully] clear solution you have in this test tube 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 the filter paper, the molecule is still small enough to pass through.*

6. Use the ice cold rubbing alcohol from an ice bath. Using a drinking straw, gently add rubbing alcohol to the top until there is about 2-3 cm sitting above the solution. The best way to do this is to put about 40 mL rubbing alcohol into your 50 mL beaker and dip the drinking straw into the alcohol and then when it is half full put your finger over the end. To add it to the test tube, SLOWLY, let it run down the side of the test tube into the DNA solution. Your goal is to have the rubbing alcohol stay on the top of the DNA solution, with as little mixing as possible.

* *The rubbing alcohol is used to extract the DNA from the onion juice. The reason you want the rubbing alcohol to stay on top of the onion juice is because by doing that the liquid will form two distinct layers. Generally, molecules are attracted to the boundaries of two liquids-sometimes the concentration of large molecules can be mush higher at the boundary between the two liquids. If the DNA is attracted to the surface, we can pull most of it 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 surfaces, making it much harder to pull any out of the tube*.

7. VERY GENTLY insert a glass rod through the upper alcohol layer in the test tube into the DNA containing the buffer solution. Your goal is to disturb the solution as little as possible. Leave the glass rod in the one place and rotate it in one direction, with luck the DNA fragments will wind onto the stick in the same way thread winds onto a spool

* *DNA spools onto the glass rod because the exposed ends have polar chemical groups on them. Glass is polar, so the ends are attracted to the stirrer. By winding the stirrer you are basically just reeling the DNA molecules.*

8. After twirling the stick for about 60 seconds, pull the stirrer up through the alcohol later. You should see the DNA adhere to the end of the stick appear as transparent sludge. The molecule you have collected on the stick contains the genetic information of the onion.’

* *When you pull the DNA through the nonpolar alcohol layer, it clumps together because it would rather be attached to polar materials such as the glass rod. Remember, “like dissolves like” meaning the polar compounds will tend to stay in polar environments. DNA, the polar compound sticks to itself simply because it prefers environments [itself] to a nonpolar environment [rubbing alcohol].*

9. Clean up: The waste left over from this lab can all go down the sink, however, wrap the onion “goo” in a paper towel and throw it in the garbage.