

## Extraction of DNA from an Onion

Molecular biologists and biochemists are involved with research in finding out as much as possible about the DNA in plants and animals. Although DNA was discovered in the 1950's, there still remains a lot to be known about it, especially how it is used to determine the physical traits that we all have, and how it regulates the workings of the body.

We should always remember that DNA is just a chemical named *deoxyribonucleic acid*. Because it is a chemical, we can do reactions with it just like we can work with any other chemical. In this lab, we will use the chemical properties of DNA to extract it from the cells of onions.

### **Experiment:**

Note: You should write all observations from this lab in the observation section on the third page of this lab. These observations will account for a large part of your grade, so be neat and complete!

- 1) Prepare a buffer solution by pouring the following into a clean 250 mL Erlenmeyer flask:
  - 120 mL of water (distilled water, if available)
  - 1.5 grams of sodium chloride (table salt)
  - 5.0 grams of baking soda (sodium hydrogen carbonate)
  - 5.0 mL of shampoo or liquid laundry detergent

*What buffer solutions are used for:*

*This buffer solution is used in this lab for several reasons. First of all, 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.*

*Secondly, 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 in your clothes, it will also work to tear apart the "greasy" molecules in cell walls. It will be important that these cell walls break down in this lab, because inside the cell is where the DNA is.*

- 2) Chill the buffer solution by placing the flask in a larger beaker filled with crushed ice and water.

*Why we need to chill the buffer solution:*

*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 the DNA breaks up, we cool down the buffer solution to near 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 take place.*

- 3) Dice an onion with a knife. Half an onion should be plenty for this lab. Use a mortar and pestle to mash the pieces of onion into a pulpy sludge. If you don't have a mortar and pestle, 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!

*Why we need to mash the onion:*

*What we want to do by mashing the onion is to either break the cell walls (releasing the DNA into the juice) or at the very least expose the cell walls so the detergent can break them down.*

- 4) Place 10.0 mL of the vegetable mush/juice into a small, clean beaker or flask and mix in 20 mL of the chilled buffer solution. Stir vigorously with a stirring rod for three minutes.

*In this step, we're 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 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.
- 6) Making sure to leave the pulp behind, strain the rest of the solution through a coffee filter or filter paper with a funnel and 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 a piece of filtering paper, the molecule is still small enough to pass through.*

- 7) Obtain some ice-cold rubbing alcohol from an ice bath or a freezer. Using a drinking straw, gently add rubbing alcohol to the top until there is about an inch and a half sitting above the buffer solution. The best way to do this is to dip the drinking straw into the isopropanol bottle and then when it has filled to 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 much higher at the boundary between two liquids. That's what we're hoping for here... if the DNA is attracted to the surface, we can pull most of it out. However, if the alcohol and onion juice mixes 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) 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 one direction, like you would spin a globe; with luck the DNA fragments will wind onto the stick in the same way that thread winds onto a spool.

*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. You should see the DNA adhere to the end of the stick and appear as a transparent, viscous sludge at its tip. The molecule that you have collected on this stick consists of the entire genetic code for the making of an 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 stick or even itself. Remember, "like dissolves like", meaning that polar compounds will tend to want to stay in polar environments while nonpolar compounds will want to stay in nonpolar environments. In this case, DNA, a polar compound, sticks to itself simply because it prefers a polar environment (itself) to a nonpolar 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, 10 mL of isopropanol solution, and a large amount of onion goo. All solutions can go down the sink, while the onion goo should be wrapped in a paper towel and thrown in the garbage. The collected DNA is a totally safe compound, and can be thrown away or saved to show your parents and friends if you like.

**Observations:** Use the space here and on the back of this sheet to write down any observations that you have about this lab.

