DNA EXTRACTION: Classroom Reference

INTRODUCTION:

Deoxyribonucleic acid is an extremely long molecule found in the chromosomes of all eukaryotes. It's also found in all prokaryotes, and some viruses. In humans, a 20 μm cell has about 2 m of DNA, divided among 46 chromosomes. In order to organize and pack it all in, DNA is wound around *histones* (protein "spools").

There are many techniques to *extract* (remove) DNA from cells, but they all have the same 4 basic steps:

A. Lyse (break open) the walls and membranes. Cell walls can be physically lysed by mashing it by hand or with a mortar & pestle. Since cell and nuclear membranes are made of lipids, *surfactants* will chemically lyse the membranes. (Think about it…what do you use to wash greasy dishes? Oily hair?)

B. Neutralize charged areas. The negatively charged phosphate groups will make DNA molecules repeal each other. Salt provides Na^+ ions that neutralize this effect.

C. Destroy proteins. To release DNA, histones must be destroyed. Nuclease digestive enzymes also must be destroyed so the DNA is not damaged. Protein destruction is either done chemically (using enzymes called *proteases*) or with heat.

Proteases lyse other protein molecules. One common home example is contact lens enzyme cleaner. Another is any type of fresh fruit that cannot be used in making Jello. (Go read a Jello box! Which fruits have proteases?)

If heat is used, it must be done carefully. 50°C destroys proteins, but temperatures over 80°C will destroy nucleic acids.

D. Precipitate (solidify) the DNA. So far, DNA is dissolved in an aqueous (water) solution. However, it precipitates in alcohol and becomes a solid, visible mass. DNA is not attracted to plastic, but may stick to wood or glass.

PURPOSE: To extract and visibly observe DNA.
To see if freezing a fruit has an effect on DNA when compared to fresh fruit.

PART I: DNA From Fruits

MATERIALS (per sample):

- DNA source: fruit (fresh and frozen)
- 2 ziplock bags
- 2 funnels
- 2 filter papers or coffee filters
- 2 plastic test tube
- wooden stick (do NOT throw away!)
- 10 ml graduated cylinder or pipet
- lysis buffer (made from 5 g NaCl, 100 ml soap or other surfactant, and 500 ml H_2O)
- saturated protease solution (made by dissolving meat tenderizer in H_2O)
- cold 91% rubbing alcohol
- ring stand and 2 rings

PROCEDURES:

1. Each group will test 2 samples of the same fruit: 1 fresh, 1 frozen.
2. Place each sample into separate ziplock bags. Carefully mash fruit using fingers. Do not break the bag.
3. Add 10 ml of lysis buffer; continue to mash for 5 min.
4. Fold coffee filter into quarters, then open into a cone shape & place into a funnel.
5. If the funnel has a long stem, use the ring stand to position the funnel over a test tube.
6. Add the mashed fruit into the filter and collect filtrate until test tube is about 1/3 full.
7. Add 20 drops of protease solution to the test tube; stir *gently*.
8. Tilt your test tube and slowly add cold alcohol so that it forms a layer on top of the DNA source mixture. Keep adding until the top and bottom layers are about equal.
9. DNA will begin to precipitate and rise from the bottom layer into the alcohol layer. The wooden stick may be used to help *lift* the DNA into the alcohol, but don't stir!
10. In one tube, try to spool the DNA onto the wooden stick. If successful, your DNA is still in long strands. If the DNA cannot be wound around the stick, it has been sheared (broken)
11. Compare the relative amount and appearance of the DNA collected, and record observations from each group.

PART II: DNA From Human Cheek Cells

MATERIALS (per person):

human cheek cells
water
8% NaCl solution
25% soap solution
saturated protease solution (made by dissolving meat tenderizer in H_2O)
cold 91% rubbing alcohol
1 sm disposable cup
1 large plastic culture tube
wooden BBQ stick
1 microtube (if you want to keep *your* DNA)

PROCEDURES:

Each person will be collecting their own DNA. This extraction works best if students have not recently eaten or chewed gum.

1. Pour about 6 ml water into a cup.
2. Take the water and **violently** swish the water in your mouth, making sure to rub your tongue along your cheeks and/or chew on your cheeks for a full 30 seconds.
   The amount of swishing will actually become quite laborious – hang in there!
3. Spit the water mixture back into the cup, then pour it into a culture tube.
4. Add 10 drops of 25% soap.
5. Add 10 drops of 8% salt solution.
6. Add 20 drops of saturated protease solution.
7. Close the culture tube, then **gently** rock the tube on its side for 2-3 min.
   DO NOT BE TOO VIGOROUS; physical abuse may shear (break) the long DNA molecule into small fragments.
8. Open and slightly tilt your culture tube. Slowly add cold alcohol so that it forms a layer on top of the DNA source mixture. Keep adding until the top and bottom layers are about equal.
9. DNA will begin to precipitate and rise from the bottom layer into the alcohol layer. The wooden stick may be used to help lift the DNA into the alcohol, but don't stir!
10. In one tube, try to spool the DNA onto the wooden stick. If successful, your DNA is still in long strands. If the DNA cannot be wound around the stick, it has been sheared (broken).
11. If you would like to keep your DNA, get a microtube and add alcohol to it. Scrape/shake the DNA from the wooden stick into the microtube.