**Isolating deoxyribonucleic acids from *Fragaria virginiana* ("strawberry")**

You, a human, have 2 sets of DNA, one from each parent, in each of your cells. 23 strands of DNA from your male parent, and 23 strands from your female parent, in each of your nuclei. **2** x 23.... That means you are *diploid* [2 (n)]. Some species of strawberries, with *8 sets* of DNA, are *octoploid.* This species of strawberry has 8 sets of DNA [8 (n)]. Each set has 7 chromosomes, so each cell has 8 (7) chromosomes = 56 chromosomes. One 25 gram strawberry could have more than 5 million cells!

**NOTES on DNA isolation:** The first step in our case will be to break up the sample tissues. Some of this will be done **mechanically** (by crushing and grinding) and some **chemically** (by breaking up lipids with agents of deterrence (detergents). A second step in DNA purification is to remove proteins from the solution. This is important simply for the sake of having a pure solution of DNA, as well as preventing the action of any protein that might interact with the DNA. This is achieved by **proteases** in solution.

The next step is to **precipitate** the DNA out of solution. DNA is negatively charged due to the phosphates in its backbone. The negative charge is what allows it to dissolve in water (since water is polar, "like dissolves like"). The key to precipitating DNA is to somehow **mask** the negative charge. This can be done with sodium chloride (“salt”), which when dissolved in water, produces positive sodium ions and negative chloride ions. The goal is to have the negative DNA bind to the positive sodium and cancel the charges.

However, there is a problem. Just as water dissolved the sodium chloride into its ions, if sodium and DNA bind together, water will just turn around and dissolve them as well. This is why you add either isopropanol or ethanol (varieties of alcohol). In these alcohols, the sodium + DNA complex does not dissolve (it is **not "alcohol soluble"**) and thus **precipitates** out of solution. Since the DNA is now a solid, it will appear to "group together" (coalesce) in the alcohol solution.

If you centrifuge it, the DNA will all go to the bottom and liquid will remain on top. You can then remove all the liquid and then re-suspend the DNA in some buffer to produce a solution of just DNA.

1 What is the purpose of the sodium chloride ?

2 Why is the cold ethanol added to the soap and salt mixture ?

**Materials**

2 “zip lock” bags

strawberries (1 “medium” strawberry should be fine)

graduated cylinder

lysing solution = a solution containing: ~ 0.9 % NaCl ; papain (“meat tenderizer” : an enzyme that destroys proteins that would interfere with the DNA extraction); mild detergent/shampoo (containing sodium laurel sulfate, aka, sodium dodecyl sulfate = SDS = (C 12 H 25 SO 4 Na)) ; water

warm water bath (55oC-60oC)

ice/cold bath

test-tube/plastic tube (for holding liquids)

beaker or plastic cup and/or filter (for holding and filtering liquids/tubes)

coffee filter

ice cold ethanol (97% alcohol)

pipette (“eyedropper”)

glass spooling rod/forceps/probe

**Procedure for Extraction**

1. Obtain two small ziplock bags. Label one as your own using a piece of tape. Place this one inside the other, like a liner.

2. Place one or two strawberries into the inner most of the 2 zip lock bags. Push out the air and seal the bag. Use the rounded bottom of the test tube, your fingers, or the bottom of the beaker to **mash** the strawberries in the bag, being careful not to tear the bags. This should produce a homogenate of strawberries. Basically, the mechanical breakdown of the cell wall and overall structure.

3. Add *about* 15 ml of the **lysing solution** to the bag.

 1 How much LYSING solution is added?

 2 Why?

4. Mush again for ***one minute***. This is the chemical portion of the extraction procedure to lyse the cell membranes and *denature* the **histone proteins** to release the DNA.

 3 What is this step doing?

5. Put bag into the **hot water bath at 55oC – 60oC for about *three minutes***. Heating helps to maximize the **protease reaction** to make sure that DNA is released from the histone proteins it is bound up in as a “chromosome”. The temperature is critical to this step. DNA is denatured at temperatures near 80oC.

6. Remove the bag from the hot water bath and put the bag into the **ice/cold bath** . Let this lysate cool for about ***1 minute.***

7. You will use the filter paper provided to you for the next step. Some may receive a funnel and filter paper device, some may receive a ring stand with filter paper attached to it.

8. Filter the strawberry **lysate through the filter into a beaker.**  AFTER ALLOWING THE LYSATE TO FILTER ***FOR A FEW MINUTES (2 or more)***: You MAY ask to use the rounded bottom of a test tube (or bottom of the beaker) to HELP push the liquid (filtrate) through the mesh of the filter. **Collect this filtrate** (the liquid that went through the filter). When you are finished squeeze the filter to get the remainder of the extract or lysate. Leave the solid residue in the paper. We do not want the solid residue for the rest of the investigation.

9. **Pour filtrate/lysate into a plastic tube.** Fill the tube to about ½ the volume.

10. Use the transfer pipette to **drip alcohol slowly** down the sides of the tube, while holding the tube an angle of approximately 45o. Try to make a clear and undisturbed layer of alcohol to float on the lysate. The line between the two layers is called the interface.

11. At the interface, you will **see the DNA precipitate** out of solution and float to the top. You may spool the DNA on the glass spooling rod/forceps available to you. You should see strands of DNA winding around the rod/forceps.