**CHEM 132 Lab #5**

**Strawberry DNA**

**Introduction**

The native British wild strawberry is a "diploid" - it has two setsof chromosomes, as in humans. The most commonly cultivated strawberry, *Fragaria ananassa,* is an octoploid with eight sets. This makes it a good candidate for demonstrating DNA extraction - with eight copies of each gene in the strawberry genome, strawberries are packed full of it. The strawberry, it turns out, has a long and complicated family history. "The cultivated strawberry is interesting from a genomic perspective, because it's a polyploid hybrid species." Unlike peas, for example, or humans, for that matter, which are diploids (with two sets of chromosomes), a strawberry is an octoploid (with eight sets of chromosomes). How some strawberries evolved from diploids to octoploids is part of the story that people are trying to unravel.

Many people are surprised to find out that strawberry growers plant bare-root plants rather than seeds. The reason is every strawberry seed contains different genetic material, the product of a myriad of potential gene combinations. Because the genetics of strawberries are so diverse (humans are diploid, strawberries are octoploid). For strawberries, the mother plant puts out runners (called daughter plants) that were essentially identical to her, which in turn also put out runners. The major varieties of strawberries grown in Florida are Sweet Charlie, Camarosa, and the Oso Grande.

One of the reasons strawberries work so well is that they are soft and easy to pulverize. Also, ripe strawberries produce enzymes (pectinases and cellulases) which aid in breaking down the cell walls. Most interestingly, strawberries have enormous genomes. They are octoploid, which means they have eight of each type of chromosome (which equals abundant DNA).

**Materials needed**

* Mild Shampoo (containing Laurel sulfate, with no conditioner)
* Sodium chloride
* Papain (Meat tenderizer). This is an enzyme that destroys proteins that would interfere with the DNA extraction.
* Frozen or fresh strawberries or raspberries - about 1 to 2 cups
* Zip lock bags (double bags), Corning tubes, Beaker or plastic cup, Transfer Pipets
* Cheese cloth (Melitta Coffee filters or glass wool are an alternative)
* Rubber bands, Eppendorf tubes (Microcentrifuge tubes), Microcentrifuge
* Ice cold ethanol (97%)
* Tris EDTA solution

**Procedure for Extraction**

1. Place several strawberries in a double zip lock bag. Use the top of the Corning tube to mash the strawberries in the bag. This should produce a homogenate of strawberries. This is a mechanical breakdown of the cell wall and structure.
2. Add 15 ml of the **extraction buffer** (100 ml shampoo + 15 g NaCl +1L H2O) to the bag.
3. Mush again for one minute. This is the chemical portion of the extraction procedure to lyse the cell membranes to release the DNA.
4. Put bag into the hot water bath at 55°C - 60°C for 10 min. Heating helps to maximize the reaction to make sure that DNA is released. The temperature is critical to this step. DNA is denatured at temperatures near 80°C.
5. Remove and put bag into the ice chest for 10 min.
6. Put cheesecloth over the top of a beaker or plastic cup. Hold with a rubber band around the top.

7. Filter through cheesecloth in a funnel into beaker. Use the top of the Corning tube to push through the mesh of the cheese cloth. Collect the filtrate. When you are finished squeeze the cheesecloth to get the remainder of the extract or lysate.

1. Pour filtrate into a plastic tube. Fill the tube to about 1/2 the volume.
2. Use the transfer pipet to drip alcohol slowly down the sides of the tube, while holding the tube at approximately an angle of 45°. 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.
3. At the interface, you will see the DNA precipitate out of solution and float to the top. You may spool and collect the DNA on your glass rod, wooden stick or cotton swab. The fibers are millions of strands of DNA winding around.
4. To view in a microscope, put the glob on a clean slide and gently tease/stretch apart using 2 toothpicks or dissecting pins. The fibers will be easier to see in the teased-apart area.

**Explanation**

* The detergent in the shampoo helps to dissolve the phospholipid bilayers of the cell membrane and organelles.
* The salt helps keep the proteins in the extract layer so they aren't precipitated with the DNA.
* DNA is not soluble in ethanol. When molecules are soluble, they are dispersed in the solution and are therefore not visible. When molecules are insoluble, they clump together and become visible. The colder the ethanol, the less soluble the DNA will be in it yielding more visible "clumping". This is why it is important for the ethanol to be kept in a freezer or ice bath.

**Preparation of DNA for Isolation and Purification**

1. Put the DNA in an Eppendorf ( microcentrifuge tube) (1.5m1)
2. Spin the Eppendorf ( microcentrifuge tube) for two minutes.
3. Pour off the liquid in the tube. This leaves a pellet in the bottom of the tube which is DNA.
4. Add 25 - 50 µl of Tris EDTA Buffer to the Eppendorf tube. Resuspend the pellet of DNA in the buffer. This makes the DNA go into solution.

**Data Sheet**: Strawberry DNA

Answer the following questions in complete sentences.

1. What steps in the procedure contributed to the release of the DNA from the Strawberry plant cells?
2. What temperature is used to speed the extraction of DNA?
3. Why is ethanol used for the precipitation or isolation of DNA?
4. How is the actual DNA separated from the rest of the lysate?
5. Describe the appearance of the DNA.

**Pre lab**: Strawberry DNA

1. Name the 3 reasons the strawberries are an excellent source of DNA.
2. What ingredients are in the extraction buffer? What is the role of buffer in the extraction of DNA?