DNA Extraction Lab

Background Information:

Almost every one of our cells contains DNA. DNA is the genetic material in the cells of all living things. Bacteria are the only prokaryotes on Earth that do not have their genetic material contained in a nucleus. Plants, animals, fungi, and protists are eukaryotic since they have membrane bound organelles and a true nucleus. DNA is the code of life: it is the hereditary material and codes for the production of proteins in every organism. In fact, the genetic information of all living things is in the form of DNA—only the sequence of bases (A, G, C, T) is different. While you can't see a single molecule of DNA without a very high-power microscope, if you take the DNA from many cells and mass it together, it looks like strands of translucent cotton.

In this activity, you will be trying to extract the DNA from fruit. Plant and animal cells are very similar, but with a few key differences. In both cases, the DNA is located in the nucleus. To get at it, you have to break down the barriers of the cell membrane and the nuclear membrane. In plant cells, there is an additional cell wall to break down. Breaking open a cell is called cell **lysis**. First, you can physically crush the cells to break the cell wall. Then you can use a detergent-based extraction buffer (a combination of shampoo, salt, and water) to disrupt/ destroy the cell and nuclear membranes. Just like dish detergent breaks down fats (lipids) on a frying pan, the detergent-based extraction buffer breaks up the phospholipids in cell membranes, creating a **lysate** or cell extract. The salt will cause the proteins and carbohydrates of the cell to precipitate out and finally the alcohol is used to extract the DNA out of the mixture.

The strainer filters out the solid debris, leaving only the DNA in the solution. Like salt, DNA is soluble in water. DNA is not soluble in alcohol. Keeping the solution cool by using an ice bath helps prevent natural enzymes from destroying the DNA once it is no longer protected by the membranes of the cell. Cooling the alcohol maximizes the amount of DNA that we are able to extract. When ethanol is added, the DNA starts to precipitate. You can then "spool" the DNA around a glass stirring rod. Strawberries work well for this activity because they are octoploid, meaning they have eight copies of each of their seven chromosomes in every cell (humans are diploid: two copies of each, one each from mom and dad).

Purpose:

To extract DNA from strawberry cells and isolate it for gel electrophoresis.

Materials:

Strawberry	Extraction Buffer	Micropipette	Strainer
Large Test Tube	Erlenmeyer Flask (250 ml)	Ethanol	Ice Bath
Glass Stirring Rod	Graduated Cylinder (50 ml)	Ziploc Bag	

Procedure:

1) Place a strawberry into your zip-lock bag, slowly work the air out of the bag, and seal it.

2) Gently mash the strawberry (don't burst the bag!) for 2 minutes.

3) Using a graduated cylinder, pour 15 ml of extraction buffer into the bag, reseal bag, and mix the strawberry and buffer together for one minute.

4) Place the collecting tube into the Erlenmeyer flask to keep it steady. Put the strainer over the funnel and carefully pour the strawberry extract through the funnel into the collecting tube. Gently squeeze the residue in the strainer to get some of the liquid that remains in the pulp. Discard the plastic bag and clean the strainer and funnel.

5) Using a micropipette obtain 5 ml of ice-cold ethanol. *Slowly* add 5 ml of ice-cold ethanol to the tube so that it runs down the inside of the tube onto the extract.

6) Watch closely as DNA begins to clump where the two layers meet. Take the glass stirring rod and place the end in the top layer, just above where the layers meet.

7) Slowly twist the glass stirring rod as you wind the DNA onto the end of the stick. Remove the rod and observe the DNA.

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Data/Results:

Describe your observations ***before and after*** adding the ethanol to the tube.

Compare the amount of DNA you got from the fresh vs. the frozen strawberry. Which one do you think you got more DNA from?

Discussion:

1) What did the DNA look like? Relate its chemical structure to how it looked when lots of it precipitated on the stick.

2) Considering what you know about cell structure and the location of DNA, suggest a reason why a detergent-based extraction buffer was used in this lab?

3) Why did we use ethanol in this experiment?

4) What do you think might have happened if the ethanol was added too quickly and the two layers mixed?

5) What could account for the difference in the amount of DNA you got from the fresh vs. the frozen strawberry? Name two and explain each.