

Biology 3A Laboratory

DNA Isolation and Quantification

- Isolation of DNA from plant products.
- Determine which items yield more DNA.
- Understand the physical and biochemical properties of DNA in living organisms.
- To quantify the amount of DNA from an unknown using spectral analysis.

Part A: Isolation and Purification of DNA from Vegetables

The isolation and purification of DNA is the starting point for nearly all genetic engineering experiments. In order to extract DNA from a cell, it is necessary to break open the cell and the cell's nucleus such that the DNA is released into solution (grind and find). To do this extraction, a homogenation buffer with a detergent is required to break up the cell and nuclear envelop. This is also a stabilizing buffer with salt and other compounds to keep the pH constant and keep the nucleic acid intact. In this procedure, you will chop up the tissue, treat the chopped tissue with heat and detergent to break up the membranes, further macerate (grind up) tissue to release the DNA and precipitate the DNA with cold ethanol (find).

Procedure A: (modified from "An Introduction to Plant Science", Arnoldsen, Gilroy and Krupnick, 1999):
Work in groups of 4 on this experiment for steps 1 through 5 and as pairs for the remaining steps.

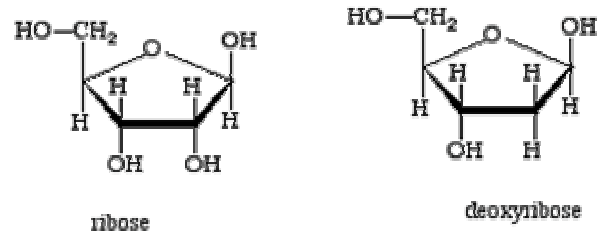
1. Obtain a vegetable as directed by your instructor and mince the vegetable into small pieces (the smaller the better). We have only 5 cutting boards and knives, so please share.
2. Place 50 g of the chopped vegetable into a 400 ml beaker and add the homogenation buffer to a volume of 200 ml of soap/salt solution (100 mL Woolite, 15 g NaCl, 900 mL deionized water).
3. Place the beaker with the chopped vegetable and buffer into a warm water bath (60° C) and let it incubate for 15 minutes.
4. Remove the beaker from the water bath and place it in an ice bucket. Let the solution in the beaker drop to 23° C.
5. Dump the cooled contents of the beaker into a pre-chilled blender. Blend the solution for 1 minute at low speed and at 1 minute at high speed.
6. Pour the ground (homogenized) vegetable tissue into two 400 mL beakers such that the solution is equally divided between the two beakers.
7. Let the ground tissue stand for 30 minutes at room temperature. During this time, prep the materials for the spectral analysis of DNA (or do something else that's constructive).
8. After 30 minutes, filter the homogenated tissue through cheesecloth into another beaker. Collect only the clear solution and leave the foam behind.
9. Remove 5 mL and place into a test tube to be used for the spectral analysis. Place way from any heat source.
10. Place the beaker into an ice bath. Add 100 mL cold ethanol. This will cause the DNA to precipitate.
11. Collect the precipitate by spooling it onto a glass rod. The long fibrous goop is purified DNA.
12. Observe the other DNA from the other groups and make a comparison.

Part B: Spectral Analysis of DNA

In part A, you isolated DNA from a particular tissue. In the exercise, you will quantify the amount of DNA from your plant tissue. DNA and RNA are nucleic acids made of nucleotide subunits (Figure 1). One major difference between DNA and RNA is their sugar: DNA contains deoxyribose, whereas RNA contains ribose. DNA can be identified chemically with the Dische diphenylamine test. In this test, acidic conditions convert

deoxyribose to a molecule that binds with diphenylamine to form a blue complex. The intensity of the blue color is proportional to the concentration of DNA.

Figure 1: Comparison of ribose and deoxyribose.



Procedure B:

1. Obtain seven test tubes and number them 1-7.
2. Add the materials listed in Table 1.

Table 1: Test tube contents for DNA spectral analysis

Tube No.	Contents
1	3 mL of 1 mg/mL DNA
2	3 mL of 0.5 mg/mL DNA
3	3 mL of 0.25 mg/mL DNA
4	3 mL of 0.125 mg/mL DNA
5	3 mL of DI water
6	3 mL of sample

3. Add 3 mL of the Dische diphenylamine (**this is a very strong acid – use appropriate equipment and use under the fume hood**) reagent to each tube and mix thoroughly.
4. Place the tubes in a boiling water-bath to speed the reaction.
5. After 10 min, transfer the tubes to an ice bath. Gently mix and observe the color of their contents as the tubes cool. Record your observations in Data Table 1.
6. Determine the absorbance for each sample at A_{600} and record them on your data table.
7. Discard all solutions with Dische diphenylamine in the **waste container** under the fume hood.
8. **Make all calculations before you leave the lab and enter your raw data (from question #12) on the computer before you leave.**
9. All data will be compiled for all laboratory sections. Please download from the class webpage.

Part A: DNA Isolation

1. Record the exact weight of plant tissue you started with: _____
2. Record your observations of the DNA extraction solution at the following steps:
 - a. After the homogenate buffer and heating to 60 °C.
 - b. As ethanol and aqueous layers are being “stirred” together.
 - c. Appearance of DNA spooled onto glass rod.
3. It is apparent that the plant cells contain a huge amount of DNA. How can so much DNA fit into the nucleus of a plant cell?
4. What was the function of the detergent in the homogenation buffer?
5. Which of the plant materials yielded the best results? Why do you think that plant material worked the best?
6. Briefly describe the appearance of the spooled DNA for the various plant materials.

7. Does the quantity of the DNA differ between the onion, the peas, banana and carrot? Do you think the actual structure of the DNA molecules differs among the three test organisms?

8. Grapes have a lot of DNA in them. However, when you used the same protocol, you did not obtain any DNA from the grapes. What can you do differently so that you could get DNA from your grapes?

Part B: Spectral Analysis of DNA

9. Data Table 1: Results from the spectral analysis of DNA

Tube No.	Contents	Color after boiling	A ₆₀₀
1	3 mL of 1 mg/mL DNA		
2	3 mL of 0.5 mg/mL DNA		
3	3 mL of 0.25 mg/mL DNA		
4	3 mL of 0.125 mg/mL DNA		
5	3 mL of DI water		
6	3 mL of sample		

10. Using Excel, graph the absorbance for the four standard DNA samples. Then determine your sample's concentration based upon this.

11. Calculate (*show your work below*) your DNA concentration using the following formula and compare it to the concentration from question #10. You may use any of the standard DNA concentrations to do this calculation.

$\frac{A_{\text{your DNA}}}{A_{\text{standard DNA}}} = \frac{\text{Concentration}_{\text{your DNA}}}{\text{Concentration}_{\text{standard DNA}}}$

For example: Say $A_{\text{your DNA}} = 0.095$, $A_{\text{standard DNA}} = 0.500$

Conc : $0.095/0.500 \times 0.25 \text{ mg/mL} = 0.0475 \text{ mg/mL}$

12. Calculate the amount of DNA yielded from your sample. Take your calculated concentration from question #11 and multiply it by the volume and convert to grams. **Show your work below for both mg and g values.** *For example: $0.0475 \text{ mg/mL} \times 2 \text{ mL} = 0.095 \text{ mg}$*

13. Calculate the % of the weight of the onion tissue that was DNA using the formula (*show your work*):

$$\% \text{ DNA} = \frac{\text{gram of DNA}}{\text{gram of plant material}} \times 100$$

14. Using the combined laboratory data, run a t-test for your plant material's DNA versus another plant's DNA.

Data will be available from the class webpage.

- What hypothesis are you testing?
- Is this a one-tailed or a two-tailed t-test?
- Construct a figure for your comparison.