

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.

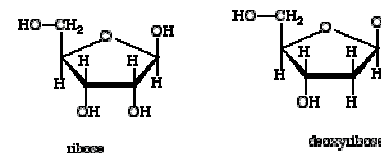
1. Obtain a vegetable as directed by your instructor and mince the vegetable into small pieces (the smaller the better). We have only 5 cutting boards and knives, so please share.
2. Place 50 g of the chopped vegetable into a 400 ml beaker and add the homogenation buffer to a volume of 200 ml of soap/salt 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. **UNDER THE FUME HOOD!** 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 IN THE BACK OF THE ROOM.
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 FOR STATISTICAL ANALYSIS.