Biology 3A Laboratory **Name:** _____
DNA Isolation Worksheet

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. After all groups have extracted the DNA, 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. **THIS IS A CONCEPTUAL UNDERSTANDING QUESTION**: 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 x 0.25 mg/mL = 0.0475 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 g and mg values.** *For example: 0.0475 mg/mL x 2 mL = <u>0.095 mg</u>*

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

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

14. Using the combined laboratory data, run the **appropriate** t-test for your plant material's DNA versus another plant's DNA. Data will be available from the class webpage for you to download.
    - What hypothesis are you testing?
    - In the figure caption you will need to state:
        i. The appropriate t-test
        ii. Is this a one-tailed or a two-tailed t-test?
    - Construct an appropriate figure for this comparison.