J. Harwood
Howe Sound Secondary School
B205

Name:

DNA Extraction Inquiry Lab **/45**

Purpose

- To review the scientific method, experimental variables, and lab procedures
- To research, develop, and implement a DNA extraction procedure
  - You will test how much the *manipulation of materials or procedure* affects DNA yield.
- Extract as much DNA as possible from a fruit or vegetable of your choice

Hypothesis

- An experimental hypothesis should refer to the independent and dependent variables, plus include a theory to justify the inference
  - i.e. If we (independent variable change), then (dependent variable prediction), because (your theory or "why").

Safety

- What safety considerations do you need to make to perform YOUR lab

Materials

Materials	Useful Property
Various fruits and vegetables	Source of DNA
Knives, cutting boards, blenders, mortars and pestles	Mash up vegetables to make them easier to work with, and to start to break up cell walls and membranes to release DNA
Various brands of dish detergents	Breaks down cell membranes by disrupting hydrophobic and hydrophilic bonds. Detergents also form complexes with lipids and proteins, causing them to precipitate out of solution.
Salt (iodized and non-iodized)	Salt helps to disrupt cell walls and membranes, and also shields the negative phosphate end of DNA, helping DNA to precipitate out of solution when alcohol is added. It also helps proteins and carbohydrates to precipitate out of solution.
Kettle (hot water bath)	Heat can help to denature DNA-degrading enzymes (like DNAase), but if it is too hot, it will degrade the DNA.
Ice (cold water bath)	Cold temperatures can slow down the activity of DNA-degrading enzymes to protect the DNA.
Various meat tenderizers, contact lens solution, and pineapple juice	Contain the enzyme papain, which breaks down proteins in membranes, and proteins that bind to and contaminate DNA.
Filter paper, coffee filters, cheesecloth, kitchen strainer (sieve)	Filter out large chunks of mashed-up vegetables and precipitated molecules.
Alcohols (70% ethanol, 95% ethanol, rubbing alcohol)	DNA is insoluble in alcohol, especially when it is cold.
Stirring rods, paper clips, Pasteur pipettes	Used to spool DNA.
Centrifuge	Spins solutions very quickly to cause DNA to precipitate on the bottom of the centrifuge tube.

Other materials available for use:

- ice
- plastic spoons
- paper towels
- beakers of varying sizes
- test tubes (plastic and glass)
- eyedroppers
- balance
- kettle
- thermometers
- watch glass
- weigh boat
- funnels
- items you provide yourself

Procedure

- You will be designing your procedure independently. Begin by researching what others have done in the past, and then modify it to suit your needs, materials available, and your independent variable.
- Procedure should be numbered and provide exact quantities (i.e. just like a recipe)
- You must justify each step of your procedure
  - Begin by thinking about eliminating the obstacles between where you're starting and where DNA is located within the cell
  - Choose materials based on their useful properties
  - You will need to repeat your procedure three or more times, changing only your independent variable, in order to test which procedure optimizes your DNA yield

Observations

- You must track your procedure using an observations table.
- You should be making quantitative (i.e. numbers-based) and qualitative (i.e. qualities-based) observations for each trial, and then comparing them to determine which procedure produced the greatest DNA yield.

Conclusion

- A brief summary of your results
- Does your data support or refute your hypothesis? Why or why not?
- Was your experiment "fair"? Were mistakes made, did you stick to your procedure, what could have been done better?
- In an ideal procedure, what results would you have expected and why?



When you are done you need to provide:

1. Your complete and signed (by Mr. Harwood) pre-lab activity. These notes can be written directly on the page or separately. They must be legible and logical.

2. A formal lab report. These reports may be typed or written, however they must be clear, logical, and follow the correct order. Your final report should contain:

- Purpose*
- Hypothesis
- Safety*
- Materials*
- Procedure
- Observations*
- Conclusion
- Starred items may be the same as lab partners, those without cannot.

DNA Extraction: Pre-Lab Activity

Answer the following questions before designing your procedure and get your teacher to check that you have completed this worksheet before starting your procedure.

1. To extract DNA, you will need to release it from the cell.
    a. Where is DNA located in the cell? _____
    b. What barriers will need to be broken so that the DNA in *plant* cells?
        i. _____
        ii. _____
        iii. _____
    c. What materials could be used to break through these barriers?

    _____
    _____

2. DNA will also have to be separated from other molecules present in the cell
    a. What two types of macromolecules are present in cell membranes that will need to be separated from DNA when isolating for DNA?
        i. _____
        ii. _____
    b. How can these other macromolecules be removed?

    _____
    _____
    _____

3. DNAase is a specific example of a molecule in the cell that must be removed.
    a. What is the function of DNAase in the cell (https://en.wikipedia.org/wiki/Deoxyribonuclease)?

    _____
    _____
    _____

    b. How could the presence of DNAase cause a problem when extracting DNA? Why is DNA especially vulnerable to this enzyme during this procedure?

    _____
    _____
    _____

    c. How could you avoid any problem caused by DNAase?

    _____
    _____
    _____

4. Draw a diagram to show how detergents interact with lipids and proteins. You will need to research this one.

5. The DNA must be separated from the cell solution and quantified.
    a. How will you precipitate the DNA so that it can be extracted?

    _____
    _____
    _____

    b. How will you extract the DNA once it has precipitated?

    _____
    _____
    _____

    c. How will you quantify the amount of DNA extracted (i.e. DNA yield)?

    _____
    _____
    _____

6. To optimize your procedure for DNA extraction, you will need to see how changing at least one variable affects the yield of your DNA. List five variables that you could choose to optimize.

    _____
    _____
    _____
    _____

7. When optimizing your procedure, it is good practice to change only one variable at a time. Why?

    _____
    _____
    _____

Using the information from the answers to the questions above, work in groups of 1 – 3 to design a procedure to extract DNA from one or more of the provided fruits and vegetables. Make sure you indicate which variable you will optimize, and how you will change that variable in different trials.

Teacher Sign Off: Pre- lab worksheet is complete ☐

DNA Extraction: Marking Scheme **Name:**

Lab Skills Checklist:

E = Excellent, G = Good, F = Fair, NI = Needs Improvement

Lab Skill Checklist	NI	F	G	E
Efficient use of class time				
Focused on task at hand				
Adapted experimental procedure when needed				
Appropriate use of apparatus and materials				
Conducted the experiment safely				

Marking Scale: **/45**

Pre-lab complete and accurate	0 1 2 3 4 5 6 7 8 9 10
The hypothesis is refers to both independent and dependent variables, plus provides a theory	0 1 2 3
The procedure is a reasonable method to answer the initial question.	0 1 2 3
The procedures used in this experiment follow a logical sequence and are written in numbered steps. Explanations are provided for each step.	0 1 2 3 4 5 6 7 8 9 10
The experimental procedure is complete and clear enough that another person could carry it out.	0 1 2 3
The observations table is well-organized, thorough, and easy to decifer	0 1 2 3
The conclusion is well-organized, thorough, provides adequate explanation and is complete	0 1 2 3 4 5 6 7 8 9 10
The experiment write-up is neat, presentable, and well organized (typed or hand-written)	0 1 2 3