**DNA Extraction Inquiry Lab** **Essentials of Biotechnology**

**Driving Question**: How can you use the properties of cellular structure and function to design and conduct an experiment to isolate DNA from an unknown source?

**Background and Objectives**: Scientists routinely conduct DNA extractions as the first step of more advanced biological procedures and applications. From a pure DNA sample, it is possible to screen a newborn child for genetic diseases, design new organisms, insert vaccinations into bananas, learn about ancient human ancestors, and even build super biocomputers. The process of extracting DNA involves the separation and purification of DNA from all other components of the tissue and cells. In this investigation, you will collaboratively explore various protocols and methods of DNA extraction through the process of inquiry. You will be required to relate cell structure and function to each step performed in the extractions, in order to develop your own protocol for an unknown tissue sample. Your group is encouraged to explore multiple extraction methods to discover the optimal steps and conditions for isolating the DNA. The more extractions completed, the more your group will become familiar with the overall process. Groups should collect detailed qualitative data for each extraction and consider the underlying purpose and mechanism involved during every step. Ultimately, your group will be required to justify each step involved in your final protocol. Please complete the five pre-lab questions prior to beginning the investigation.

**Pre-Lab Questions**:

P1. Read through each of the four extraction protocols listed below. You will notice several steps that are similar among the different extraction protocols. For each repeated or common step, propose a reason or mechanism for that step using your knowledge of cell structure and function.

P2. Is DNA a hydrophilic or hydrophobic molecule, please explain your reasoning?

P3. Compare and contrast DNA in prokaryotes and eukaryotes. How might these differences impact the methods used to extract DNA from these two cell types?

P4. Sketch and label a diagram of the phospholipid bilayer of the cell membrane. At minimum, label a component from each of the four macromolecules of life. Describe what is meant by the term “fluid mosaic model”, which is a term often used to describe the cell membrane.

P5. Create a data table to collect and organize qualitative information about the four extractions. You will use these data to design your final protocol for the unknown sample. Here are some questions to consider as you plan your data table: What does the DNA precipitate look like? How much DNA was present? What are some characteristics of the tissue/cells you extracted (i.e., cell wall, nucleus, etc.)? What might have lysed the cell to expose the DNA? What may have caused the DNA to precipitate out of solution?

## Onion DNA Extraction

**Materials:**

- fresh onions
- graduated cylinders (10mL and 100mL)
- plastic knife
- 15 mL test tube
- blender
- test tube rack or 250 mL beaker
- strainer
- glass stirring rod
- coffee filters
- non-iodized salt
- meat tenderizer (non-seasoned)
- dish detergent

- beaker
- distilled water
- ice cold 91% isopropanol

**Detergent/salt solution**

- 20 mL detergent
- 20 g non-iodized salt

**5% meat tenderizer solution:**

- 5 g meat tenderizer
- 95 mL distilled water

**Protocol:**

1. Cut a 2-cm square out of the center of an onion. Place the piece in a blender.
2. Add 100 mL of detergent/salt solution.
3. Blend on high for 30-60 seconds.
4. Strain the mixture into a beaker using a strainer with a coffee filter.
5. Add 20-30 mL of meat tenderizer and stir to mix.
6. Place 6 mL of the filtrate into a test tube.
7. Pour 6 mL of ice cold isopropanol carefully down the side of the tube to form a top layer.
8. Let the mixture sit undisturbed for 2-3 minutes.
9. The DNA will float, or precipitate, into the alcohol layer. If there is copious DNA in the extraction, it will appear as a translucent, thread-like substance that resembles wet cotton.

## Wheat Germ DNA Extraction

**Materials:**

- 250 mL beaker
- baking soda (optional)
- hot plate
- natural meat tenderizer (non-seasoned)
- non-roasted (raw) wheat germ
- ice cold 91% isopropanol
- thermometer
- 15 mL test tube
- pH meter (optional)
- glass stirring rod
- dish detergent
- distilled water
- test tube rack or 250 mL beaker
- graduated cylinders (10mL and 100mL)

**Baking soda solution (optional)**

- Add baking soda to distilled water until a pH of approximately 8.0 is reached.

**Protocol:**

1. Add 100 mL of distilled water to a beaker and heat to 50-60 ºC.
2. Add 1.5 g wheat germ and mix until dissolved.
3. Add 5 mL of detergent. Maintain 50-60 ºC temperature and stir for 3-5 minutes.
4. Add 3 g of meat tenderizer.
5. Add baking soda solution to bring the pH to approximately 8.0 (optional).
6. Maintain the 50-60 ºC temperature and stir for 5-10 minutes.
7. Remove from heat.
8. Add 6 mL of the solution to a test tube and cool to room temperature.

9. Pour 6 mL ice cold isopropanol carefully down the side of the tube to form a top layer.
10. Let the mixture sit undisturbed 2-3 minutes.
11. The DNA will float, or precipitate, into the alcohol layer. If there is copious DNA in the extraction, it will appear as a translucent, thread-like substance that resembles wet cotton.

## Bacteria (lima bean) DNA Extraction

**Materials:**

- dry lima beans
- dish detergent
- centrifuge
- distilled water
- centrifuge tube
- fresh papaya juice
- graduated cylinder (10mL)
- non-iodized salt
- granulated sugar
- pipette
- Epsom salts
- 15 mL test tube
- Bufferin (buffered aspirin; 325mg)
- test tube rack or 250 mL beaker
- ice cold 95% isopropanol
- glass stirring rod

**Prep buffer solution:**

- 57 g granulated sugar
- 3 g Epsom salts
- 1 buffered aspirin
- add distilled water for a volume of 500 mL

**50% detergent solution:**

- 20 mL detergent
- 20 mL distilled water

**Salt solution:**

- 30 g non-iodized salt
- add distilled water for a total of 250 mL

**Protocol:**

1. Add 14 mL of the bacterial suspension to a centrifuge tube and spin in a balanced centrifuge for 5 minutes.
2. Pour off the liquid (supernatant) and discard. You want to keep the pellet, as this has your cells.
3. Add 5 mL of prep buffer and resuspend your cells with a pipettete.
4. Add 1 mL 50% detergent solution.
5. Add 1 mL papaya juice.
6. Add 2 mL salt solution and shake for 2 minutes.
7. Place the tube in the centrifuge and spin for 5 minutes. Make sure the centrifuge is balanced.
8. Draw off 7 mL of the supernatant (liquid) as this now has the DNA and place it in a clean test tube.
9. Pour 7 mL of ice cold isopropanol carefully down the side of the tube.
10. Let the mixture sit undisturbed 2-3 minutes on ice.
11. The DNA will float in the alcohol. Gently invert the test tube to mix the interface of the two layers. You may see some tiny threads of DNA but are more likely to see fluffy, white sheared DNA.

**Beef Liver DNA Extractions**

**Materials:**

- fresh beef liver
- blender
- beaker
- sugar
- pipette
- centrifuge tube with cap
- Bufferin (buffered aspirin; 325mg)
- plastic knife
- graduated cylinders (10mL,100mL)
- Epsom salts
- distilled water
- centrifuge
- 95% ice cold isopropanol
- 15 mL test tube test tube rack or beaker
- dish detergent
- non-iodized salt

**Prep buffer solution:**

- 57 g granulated sugar
- 1 buffered aspirin
- 3 g Epsom salts
- add distilled water for a total of 500 mL

**10% detergent solution:**

- 90 mL distilled water
- 10 mL dish detergent

**Salt solution:**

- 30 g non-iodized salt
- add distilled water for a total volume of 250 mL

**Protocol:**

1. Cut out a 3x3-cm piece of liver and place in the blender.
2. Add 100 -150 mL prep buffer and 10 mL detergent solution to the blender.
3. Blend for 1 minute or until the mixture is smooth.
4. Pour the mixture into a beaker.
5. Transfer 1 mL of the mixture to a centrifuge tube.
6. Add 2 mL of salt solution, cap, and shake for 2 minutes.
7. Centrifuge for 3 minutes in a balanced centrifuge.
8. Carefully remove the tube from the centrifuge and note the two layers. The lower layer has a pellet and cellular debris and the upper layer (supernatant) has the DNA in it.
9. Pipette or carefully pour the supernatant into a clean test tube.
10. Pour 5 mL ice cold isopropanol carefully down the side of the tube to form a layer.
11. Let the mixture sit on ice undisturbed for two minutes.
12. The DNA will precipitate in the alcohol. The DNA of the liver will appear as long thread-like structures.

**Post Lab Questions**:

Q1. Throughout the various extraction protocols, temperature was a factor. Propose an explanation for how temperature (i.e., cold isopropanol, warm salt solutions) may affect the extraction of DNA.

Q2. Detergent is an important component in all salt-based DNA extractions, which is what you completed, in your investigation. In more advanced protocols, sodium dodecyl sulfate (SDS) is utilized rather than dish detergent. Explain why scientists use detergents like SDS to isolate DNA from cells.

Q3. Two of the protocols required the addition of Bufferin, which is a form of aspirin that also contains magnesium oxide. This compound will raise the pH of the solution. Why is it important to control the pH of the DNA extraction?

Q4**.** In several extractions, you added papaya juice or meat tenderizer to the solution. These both contain proteases, which are digestive enzymes. Explain why these proteases were necessary to obtain pure DNA.

Q5. Some lysis buffer solutions are isotonic and added only while blending or macerating the tissue. Propose an explanation for why isotonic buffers would be useful during this step of a DNA extraction?
Q6. Ethylene Diamine Tetra-acetic Acid (EDTA) is another common additive in more advanced DNA extractions. It is a chelating agent, which means it is a ligand that will readily attach to a central metal atom. With this in mind, it should be no surprise that EDTA has been prescribed as a medicine to treat lead poisoning. Develop an explanation for why scientists commonly use EDTA in DNA extractions.

**Post Lab Analysis**:

Now that you have completed all of the extraction protocols, you should have an intuition for why various reagents and conditions were required to obtain DNA from the different organisms. Take a moment to review all of the qualitative data you gathered throughout your investigation. Once you have completed a thorough review of the data, your group will receive a new organism to design and write a DNA extraction protocol. In your design, you must consider the cellular structure of each sample from previous extractions and also how the DNA is packaged and stored in different organisms (e.g., bacteria, plants, fungi, and animals). Once you have outlined a rough draft and justified each step in your protocol, your teacher will give final approval to begin the extraction. Upon completion of a successful extraction, modify and adjust your procedures accordingly and complete your write-up in your research notebooks.