DNA Extraction and Comparison Lab[1]

Enrichment Lab

Central Questions:
- What steps are required for extracting DNA out from the inside of cells?
- Can we extract DNA from plant tissues and soil?
- What can scientists do with extracted DNA?

Objectives:
- Attempt to separate DNA from plant tissue
- Attempt to separate DNA from soil, a never-living material
- Describe the appearance of DNA separated from the cell
- Relate the location of DNA within a cell to the procedures for extracting it

Related NE Science Standards:
Life Science – Heredity
- SC12.3.2 Students will describe the molecular basis of reproduction and heredity
  - SC12.3.2.b Describe the basic structure of DNA and its function in genetic inheritance

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Anticipated Length:
90 minutes

Lab Materials:
- Plant material (2 grams)
  - Suggested: Soybean leaf, stem or root
- Animal material (2 grams)
  - Suggested: beef or chicken liver
- Never-living material (2 grams)
  - Suggested: Soil – microwaved to destroy any microorganism DNA
- Cheesecloth (several pieces, 12cm x 12cm)
- Mortar and pestle
- Wooden skewer
- Fine sand
- Distilled water
- Graduated cylinder
- Funnel
- SDS/NaCl solution (10 mL)
- Ice water bath
- 70% ethanol (5 mL)
- Test-tube rack
- Scale and weigh boat/paper

[1] Protocol and Analysis Questions adopted from "Introduction to Biotechnology"curriculum. Big Foot Union High School Agriscience Department. Walworth, WI.

DNA Extraction and Comparison Lab

Enrichment Lab

Teacher Notes:

- SDS solution can be purchased from a science catalog in powder or liquid form. In liquid form, it is normally a 10% solution and ready to use. In powder form, just make a 10% solution (ex: 100mL = 90 mL of distilled water + 10g SDS powder) OR Woolite detergent can be used (SDS is basically mild soap) in a 50% concentration (ex: mix 50 mL of Woolite + 50 mL of distilled water = 100mL).
  - Note: Both the Woolite & SDS concentrations should have 1g of table salt added to the 100mL of solution to make an SDS/NaCl solution.
- Cheesecloth is available at most grocery stores.
- The wooden skewers are available at grocery stores and they are sometimes called bamboo skewers (used for kabobs).
  - Note: If DNA is seen and will not spool on the skewer, the long strands may have been broken apart during the mortar and pestle grinding step. If the students are too aggressive, they can break apart the DNA.
- 70% rubbing alcohol bought at the store can replace the ethanol if ethanol is unavailable.
  - Note: If possible, store the ethanol in a freezer or ice water bath so it is cold when the students need it.
- Sand can usually be obtained from any store that has fish tank supplies or from a store that sells large bags of sandbox sand.
  - Note: Meat tenderizer purchased at a grocery store can be used to replace the sand, or in addition to the sand. The meat tenderizer will help lyse the cell and contains enzymes that will break down additional proteins.
- Timing: Point out to students they will need to test 3 different materials. Perhaps suggest they complete all steps through Step 10 and let the test tube sit as they complete the extraction of the other materials. Then do all the spooling, Step 11, and observations, Step 12 at one time.
- The DNA can sit in the test tubes overnight after Step 10 and observations taken the next day. This will give the DNA more time to move into the ethanol

DNA Extraction Lab Observations and Analysis

Answer Key

Observation tables for the materials will be dependent on lab results. Ideally, they should see DNA in plant and animal material and not in the never-living material.

1. **Write a compare and contrast statement that analyzes the DNA Extraction Observations from the three different materials.**

   Compare and contrast statement will be dependent on lab results. Ideally, a comparison should be made between the plant and animal material and what they observe in contrast to what is seen in the never-living test.

2. **Correctly match the lab material with its corresponding function for the lab.**

   Lab Material:

   __b__ Sand
   __d__ SDS/NaCl Solution
   __a__ Cheesecloth
   __c__ Ethanol

   Function:

   a. Used to separate the large debris and materials from the cell parts and DNA in solution.
   b. Used to lyse the cells and in order to free the DNA.
   c. A liquid used to separate DNA from the rest of the cell components. DNA is not soluble in this liquid.
   d. A solution used to break down and emulsify the fat and proteins that make up the cell membrane.

3. **What additional tests could you do to determine if the material pulled from the test tube is DNA?**

   - Place the DNA back in water – it should disappear as DNA is soluble in water. Add ethanol to get it to reappear.
   - Test the pH – DNA is an acid, so it should be acidic. Get another test tube and place a small amount of water in it. Take the pH, add the DNA, and then retest the pH. Does it decrease?

   Teacher note: If available, use Diphenylamine DNA Testing Solution from Flinn Scientific.

4. **What would a scientist do next with this extracted DNA? Describe the process that would be used.**

   Many answers are acceptable. Here is one example: Isolating or extracting DNA is the first step in the analysis of the DNA. The DNA can further be broken down (with restriction enzymes) and replicated (with PCR technology) to be used in electrophoresis for DNA fingerprinting.

DNA Extraction and Comparison Lab

Student Name: _____

Background

The extraction of Deoxyribonucleic Acid (DNA) from cells and completely separating it from all other particles, a process called purification, are of primary importance to the field of science and biotechnology. Extraction and purification of DNA are the first steps in the analysis and manipulation of DNA that allow scientists to detect genetic disorders, produce DNA fragment patterns of individuals, and even create genetically-engineered organisms.

The process of extracting DNA involves the following steps.

1. The first step in extracting DNA from a cell is to lyse, or break open, the cell. One common way to lyse cells is to grind a piece of tissue along with a mild abrasive material in a mortar with a pestle.
2. After the cells have been broken open, a solution containing salt (NaCl) and a detergent, or mild soap containing the compound sodium dodecyl sulfate (SDS), is used to break down and emulsify the fat and proteins that make up the cell membrane and internal structures.
3. Next, the solution is cooled to alter the density of the water and push the DNA to the top of the solution.
4. Then ethanol is added. The addition of ethanol causes the DNA to precipitate, or separate out of solution. DNA is soluble in water, so it will not be seen in the original solution. It is also less dense than water, which aids in moving it into the ethanol. DNA is not soluble in ethanol and becomes visible as it moves into the ethanol.
5. Finally, the DNA can be removed from the test tube and used for further analysis.

Materials for DNA Extraction:

- Plant material (2 grams)
  - Suggested: Soybean leaf, stem or root
- Animal material (2 grams)
  - Suggested: beef or chicken liver
- Never-living material (2 grams)
  - Suggested: Soil – microwaved to destroy any microorganism DNA
- Cheesecloth (several pieces, 12cm x 12cm)
- Mortar and pestle
- Wooden skewer
- Fine sand
- Distilled water
- Graduated cylinder
- Funnel
- SDS/NaCl solution (10 mL)
- Ice water bath
- 70% ethanol (5 mL)
- Test-tube rack
- Scale and weigh boat/paper

DNA Extraction and Comparison Lab

Student Name: _____

Procedure

Note: Steps 1 through 12 will be repeated for each material: plant, animal, and never-living.

1. Put on safety goggles.
2. Place 2 grams of material for extraction (plant, animal or soil) in a mortar.
3. Add several grains of sand.
4. Pour 10 mL SDS/NaCl solution in the mortar.
5. Use a pestle to grind the ingredients until they form a thick fluid.
   - Note: Be careful not to over grind this mixture.
6. Place several layers of cheesecloth into a funnel and place the funnel into a test tube.
   - Note: To obtain several layers of cheesecloth, cut a piece the size you need and pull apart the individual layers. Place back together in an alternate pattern to avoid lining up all of the holes, making a better strainer.
7. Pour the contents of the mortar through the cheesecloth into a test tube until it contains at least 5 mL of the extract.
   - Note: You may need to gently squeeze the cheesecloth to remove all the fluid from the cheesecloth. You may flush the cheesecloth with distilled water to reach 5 mL in the test tube.
8. Place the test tube in an ice water bath for 5 minutes.
9. Measure 5 mL of cold ethanol in a clean graduated cylinder.
10. Remove test tube from water bath and hold the test tube at a 45° angle. Slowly pour the 5 mL of ice cold ethanol into the tube. Be careful to pour the ethanol slowly down the side of the tube.
    - Note: Do not pour the ethanol too fast or directly into the solution or it will mix the solution and take longer to see the DNA. If this happens, let it sit overnight.
11. Bend the tip of a wooden skewer and gently insert it into the test tube as far as the interface line between the water and the ethanol. Carefully and slowly move the stick in circles. This motion spools the long threads of DNA around the end of the stick.
    - Note: Spool just enough DNA so that you can see it and observe its physical characteristics.
12. Remove DNA from test tube and make observations.
13. Clean up all equipment and your work area and wash your hands before leaving the lab.

Student notes or questions about protocol:

DNA Extraction Lab Observations and Analysis

Student Name: _____

Plant Material — Observations and Notes	
Name of plant material:	
Were you able to extract DNA?	
If so, what was the appearance of the DNA you separated from the plant cells and spooled from the test tube?	
If not, describe what happened and the likely reason that you were not able to extract DNA.	

Animal Material — Observations and Notes	
Name of plant material:	
Were you able to extract DNA?	
If so, what was the appearance of the DNA you separated from the plant cells and spooled from the test tube?	
If not, describe what happened and the likely reason that you were not able to extract DNA.	

Never-living Material — Observations and Notes	
Name of plant material:	
Were you able to extract DNA?	
If so, what was the appearance of the DNA you separated from the plant cells and spooled from the test tube?	
If not, describe what happened and the likely reason that you were not able to extract DNA.	

DNA Extraction Lab Observations and Analysis

Student Name: _____

1. **Write a compare and contrast statement that analyzes the DNA Extraction Observations from the three different materials.**

2. **Correctly match the lab material with its corresponding function for the lab.**

Lab Material:	Function:
_____ Sand	a. Used to separate the large debris and materials from the cell parts and DNA in solution.
_____ SDS/NaCl Solution	b. Used to lyse the cells and in order to free the DNA.
_____ Cheesecloth	c. A liquid used to separate DNA from the rest of the cell components. DNA is not soluble in this liquid.
_____ Ethanol	d. A solution used to break down and emulsify the fat and proteins that make up the cell membrane.

3. **What additional tests could you do to determine if the material pulled from the test tube is DNA?**

4. **What would a scientist do next with this extracted DNA? Describe the process that would be used.**