DNA Extraction Inquiry Lab (Version 2; Biotech Series)

BACKGROUND: In this experiment, students will explore various protocols and methods of DNA extraction through the process of inquiry. The resulting DNA can be used for further experimentation/ investigation if desired. In order to maximize the outcomes of this learning experience, students should already have a basic knowledge of cellular structure and function. Students have already completed basic extractions from cheek cells and strawberries using a specific protocol; however, in this lab investigation students will ultimately design their own protocol for extraction from another genetic source. Students should appreciate that the process of extracting DNA from a cell is the first step for many laboratory procedures in biotechnology. Scientists must be able to separate DNA from the unwanted substances of the cell gently enough so that the DNA does not denature. Students will prepare DNA from sources subjected to mechanical, chemical and biological disruptions. A basic extraction requires that the DNA source is blended or macerated, treated with salt, detergent, and enzymes; however, there are numerous variations to this methodology depending on the source of the DNA and its desired usage. The blending disperses the tissues of the DNA source into single cells and helps to physically disrupt the cell wall in plant tissues. The salt chemically disrupts the cell membranes/walls, and enables the DNA strands to come together and the DNA molecules to precipitate out of solution into the alcohol layer. The detergent and enzymes break down the cell membranes by dissolving the lipids and proteins of the cell respectively and disrupting the bonds that hold the cell membrane together. In addition, the enzyme breaks down the chromosomal proteins, called histones, to help liberate the DNA. The detergent then forms complexes with these lipids and proteins, allowing them to be filtered out of solution, leaving the cells' DNA in the filtrate. The DNA is then precipitated in ice cold ethanol and spooled for purification and storage. Lastly, enzymes called DNAses can degrade and shear the DNA, so conditions must also be controlled to slow this degradation. The purpose of this lab investigation is to devise a detailed protocol for the extraction and isolation of DNA from one of the many sample options that are available to the class. Each group will inherently have a different protocol and groups are encouraged to explore multiple extraction methods to discover the optimal steps for extraction. Each group is required to conduct at least three different extractions from the protocols listed below; however, the more extractions that you conduct, the more you will become familiar with the overall process. After several extractions, analyze the other extractions using the summary chart and research the characteristics of the new samples that are available (e.g., yeast, bananas, fish, etc.). Based on your findings, design a new protocol for DNA extraction, run the experiment, and justify their results. You will most likely need to modify and refine your protocol to yield the most DNA. Your group needs to carefully read through this entire lab and conduct some research on your own prior to beginning your extractions. Remember to record all of your information in your laboratory notebooks and to follow the SOP for all notebook entries.

Additional Information:

Isotonic buffer: In the early steps of the DNA isolation procedure, the tissue is homogenized by placing it in a blender. The researcher does not want the cells to break open during the blending step. Therefore, the tissue is homogenized in an isotonic buffer solution. The isotonic property of the solution prevents the cells from bursting due to osmosis, and the buffer prevents the cells from being damaged by pH changes. A common isotonic buffer used in DNA isolation is buffered sucrose. This can be made by preparing a 0.31 molar (M) solution of sucrose. The formula for sucrose is: $C_{12}H_{22}O_{11}$

EDTA: Ethylene Diamine Tetra-acetic Acid (EDTA) is a chelating agent and laboratory reagent. It binds to and removes positive ions such as magnesium (Mg^{2+}) and calcium (Ca^{2+}) ions. These ions strengthen membranes by neutralizing the negative charges on the phospholipids, so removing these ions weakens the cellular and nuclear membranes. The formula for EDTA is: $C_{10}H_{16}N_2O_8$ and the suggested molar concentration is 2 mL – 6 mL of 0.5 M ETDA at a pH of 8.0

SDS detergent: Recall from your lab on lipids that detergents and soaps are molecules that break apart globules of lipids. Detergent molecules are attracted to both lipids and water, by binding to both lipid and water the detergent is able to dissolve the membranes of the cell. In other words, detergents lyse (break open) cells membranes and nuclear membranes. The most common detergent used in biology laboratories is called sodium dodecyl sulfate (SDS).

Sodium Chloride (NaCl): The detergents and the EDTA dissolve the cell's membranes, which releases the genomic DNA. But the released DNA is not pure. The cell's proteins are still present. To make matters worse, the proteins tend to form ionic bonds to the DNA, so they stick to the DNA and contaminate its purity. As a first step in separating the proteins from the DNA, a large amount of NaCl salt (which is Na^+ and Cl^- ions) is added. The Na+ ions neutralize the negative charge on the DNA, whereas the Cl- ions neutralize the positive charges on the proteins. With their charges neutralized, the proteins and DNA no longer form strong ionic bonds to one another and therefore can be easily separated.

Alcohol: After the NaCl has separated the proteins from the DNA, the final step in DNA isolation is usually to add ice cold alcohol to the mixture. The alcohol forms a separate layer on top of the cell homogenate. The DNA is not soluble in the alcohol layer, so it will form a precipitate (a mucus-like solid in the alcohol layer). The proteins, on the other hand, do not precipitate when the alcohol is added (they stay dissolved in the homogenate). The precipitated DNA can therefore be separated from the proteins by spooling it onto a rod, then the rod is used to transfer the spooled DNA into sterile water. The DNA has now been successfully isolated from the cell.

Although not "chemicals", there are two more features of most DNA isolations that help protect the DNA. The first is **coldness**: The DNA isolation procedure is usually done on ice to slow the action of cellular enzymes that that might degrade the DNA. The second is **gentleness**: DNA strands are extremely long and fragile. Therefore all steps should be done slowly and gently to avoid shearing the DNA strands.

Onion DNA Extraction

Materials:

- fresh onions
- graduated cylinders (10ml and 100ml)
- knife
- 15 ml test tube
- blender
- test tube rack or 250 ml beaker
- strainer
- glass stirring rod
- coffee filters
- non-iodized salt
- Adolph's natural meat tenderizer
- Palmolive detergent
- beaker
- distilled water
- ice cold 95% ethanol

Solutions: Detergent/salt solution:

- 20 ml detergent
- 20 g non-iodized salt
- 180 ml distilled water

5% meat tenderizer solution:

- 5 g meat tenderizer
- 95 ml distilled water

Protocol:

1. Cut an inch square out of the center of 3 medium onions. Chop and place in a blender.
2. Add 100 ml of detergent/salt solution.
3. Blend on high 30 sec-1 minute.
4. Strain the mixture into a beaker using a strainer with a coffee filter.
5. Add 20-30 ml meat tenderizer and stir to mix.
6. Place 6 ml filtrate in a test tube.
7. Pour 6 ml ice cold ethanol carefully down the side of the tube to form a layer.
8. Let the mixture sit undisturbed 2-3 minutes until bubbling stops.
9. The DNA will float in the alcohol. Swirl a glass stirring rod at the interface of the two layers to see the small threads of DNA.

Wheat Germ DNA Extraction

Materials:

- 250 ml beaker
- baking soda
- hot plate
- Adolph's natural meat tenderizer
- non-roasted wheat germ
- ice cold 95% ethanol
- thermometer
- 15 ml test tube
- pH meter
- glass stirring rod
- Palmolive
- detergent
- distilled water
- test tube rack or 250 ml beaker
- graduated cylinders (10ml and 100ml)

Solutions: Baking soda solution:

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

Protocol:

1. Add 100 ml distilled water to a beaker and heat to 50-60oC.
2. Add 1.5 g wheat germ and mix until dissolved.
3. Add 5 ml detergent. Maintain 50-60oC temperature and stir for 5 minutes.
4. Add 3 g meat tenderizer.
5. Add baking soda solution to bring the pH to approximately 8.0.
6. Maintain the 50-60oC temperature and stir for 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 ethanol carefully down the side of the tube to form a layer.
10. Let the mixture sit undisturbed 2-3 minutes until bubbling stops.
11. The DNA will float in the alcohol. Swirl a glass stirring rod at the interface of the two layers to see the small threads of DNA.

Lima Bean Bacteria DNA Extraction

Materials:

- dry lima beans
- Palmolive detergent
- centrifuge
- distilled water
- centrifuge tube
- fresh papaya juice
- graduated cylinder (10ml)
- non-iodized salt
- granulated sugar
- pipet
- epsom salts
- 15 ml test tube
- bufferin (325mg)
- test tube rack or 250 ml beaker
- ice cold 95% ethanol
- glass stirring rod

Solutions: Lima Bean Bacteria Suspension: Place 1-2 handfuls of dry lima beans in a large jar and fill halfway to the top with distilled water. Cover and sit in a warm room for 2-3 days. Culturing longer than three days often results in more DNA but it usually shears. Pour through a strainer and keep the liquid for the extractions.

Prep buffer solution:

- 57 g granulated sugar
- 3 g epsom salts
- 1 buffered aspirin
- add distilled water for a total volume of 500 ml

50% detergent solution:

- 20 ml detergent
- 20 ml distilled water

Salt solution:

- 29.2 g non-iodized salt
- add distilled water for a total volume 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 pipet.
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 has the DNA and place it in a clean test tube.
9. Pour 7 ml of ice cold ethanol carefully down the side of the tube.
10. Let the mixture sit undisturbed 2-3 minutes until the bubbling stops.
11. The DNA will float in the alcohol. Swirl a glass rod at the interface of the two layers. You may see some tiny threads of DNA but are more likely to see fluffy, white sheared DNA.

Thymus DNA Extractions

Materials:

- fresh thymus
- blender
- beaker
- sugar
- pipet
- centrifuge tube with cap
- bufferin (325mg)
- knife
- graduated cylinders (10ml,100ml)
- epsom salts
- distilled water
- centrifuge
- 95% ice cold ethanol
- 15 ml test tubetest tube rack or beaker
- Palmolive detergent
- non-iodized salt

Solutions: 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 Palmolive detergent

salt solution:

- 29.2 g non-iodized salt
- add distilled water for a total volume of 250 ml

Protocol:

1. Cut out a chunk of liver or thymus 1 inch square 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 7 minutes in a balanced centrifuge.
8. Carefully remove the tube from the centrifuge and note the two layers:
    - lower layer - pellet
    - *upper layer - liquid (supernatant) and what has the DNA in it.
9. Pipet or carefully pour the liquid into a clean test tube.
10. Pour 5 ml ice cold ethanol carefully down the side of the tube to form a layer.
11. Let the mixture sit undisturbed for a minute or two.
12. The DNA will float in the alcohol. The DNA of the thymus will be long threads that easily spool.

Questions	Onion	Wheat Germ	Bacteria	Thymus
What are the cell characteristics?				
What lyses the cell and nucleus?				
What protects the DNA?				
What precipitates the DNA?				
Amount of DNA				
Description of DNA				
Changes in protocol				

The following extensions and protocol are for Applications of Biotechnology students only (junior year).

EXTENSIONS: INVESTIGATIONS OF THE CHEMICAL AND PHYSICAL CHARACTERISTICS OF DNA: The characteristics of DNA can be investigated using either the extracted DNA or commercially prepared DNA. The following suggestions for investigations demonstrate that DNA has characteristic properties that are conserved across phylogenetic boundaries.

Concentration- If your Biotech Lab has a spectrophotometer, you can demonstrate that DNA has an optical absorption maximum at 260nm.

Viscosity- As DNA is a large linear polymer, it tends to be very viscous in solution. As the DNA molecules are progressively broken into smaller fragments, the solution will tend to become less viscous at the same concentration. The solution's resistance to being pipetted by a fine bore pipette can assess changes in the viscosity of the solution. The greater the resistance; the more viscous the solution will be. Students can investigate the effects on a solution of:

- Mechanical shearing- due to rough handling/ multiple pipettings.
- Heating- lowers viscosity as DNA becomes single-stranded as base pairs detach.
- Heating and cooling- several cycles of heating and cooling will lead to denaturation of the DNA.
- Alkali treatment- increasing concentrations of NaOH (greater than 30mM) will denature and eventually destroy the DNA molecule.
- Nuclease treatment- causes DNA degradation

Size- DNA can be run through an agarose gel and visualised by staining to allow size determination.

Enzyme Digestion Patterns- Restriction endonucleases can be used to generate DNA 'fingerprints' for viewing via electrophoresis.

Other Tests- There are simple tests for the components of DNA (purines, phosphate, deoxyribose) given at http://tipnet.taranaki.ac.nz/~mfenton. Due to the nature of the tests, they are best carried out as professor demonstrations.