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Title: DNA Extraction: Onion Cells

Objective(s)/Aim(s): Extract DNA from onion cells through a hands-on approach, separate DNA from other cellular material, collect long DNA strands, and explore their physical properties.

Introduction:

DNA serves as the molecular blueprint for living organisms. DNA extraction is important in the study and manipulation of nucleic acids. There are many techniques to extract DNA, but most involve breaking open the cell and using enzymatic reactions in order to destroy any other macromolecules that are not desired, leaving behind DNA. A solution made mostly of detergent called a lysis buffer is used to break down cells; these enzymes break apart the lipid molecules in the cell membrane and nuclear membranes. Other enzymes such as proteases and ribonucleases break down proteins and RNA. Then, finally, alcohol is used to precipitate the DNA, as water would cause the DNA to dissolve due to its polarity.

In this experiment, we used a simplified DNA extraction technique and performed onion tissue. The cell walls and cellular membrane were broken down using a homogenizing solution that contained sodium dodecyl sulfate (SDS), sodium chloride (NaCl), and EDTA-Na EDTA-Na₂. The DNA was heated in a thermal bath (60°C) and then isolated through precipitation with iced ethanol. Once the DNA is isolated, we use a hooked pipette to collect and observe.

Materials:

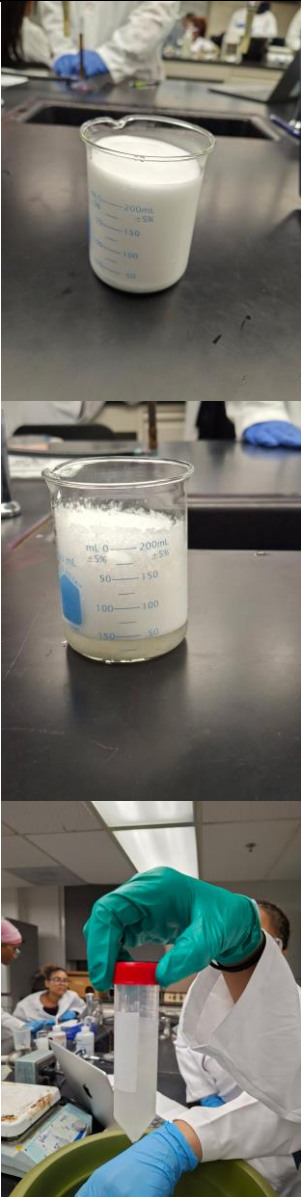
- Onion (25 g diced)
- 50 ml Homogenizing Solution (0.15 M NaCl, 0.1 M EDTA-Na₂, 1% SDS)
- 20 ml Ice-cold 95% ethanol
- Blender
- 2 Pasteur Pipet (Hooked pipette)
- Scalpel
- Balance
- 1 250 mL Beaker
- 1 500 mL Beaker
- 60°C water bath

- 1 Ice bath
- Cheesecloth and Funnel
- Thermometer
- 2 50 mL Conical Centrifuge Tubes

Methods:

1. 25g of diced onions (0.5-1cm cubes) was combined with 50 mL of the homogenizing solution in a 250 mL beaker and stirred gently.
2. The mixture of onion and homogenizing solution was placed in a 60°C water bath for 15 minutes to soften the cell walls.
3. The beaker was then transferred to an ice bath and left to cool until it reached 15-20°C, then poured into a blender and blended at medium speed for 60 seconds.
4. The blended homogenous mixture rested in an ice bath for 15 minutes and was then filtered through a 4-layer cheesecloth into a clean beaker to avoid the additional foam.
5. 10mL of the filtered extraction was placed in a 50 mL conical centrifuge tube and chilled until it was between 0-15°C. 10 mL of ice cold 95% ethanol was then poured down the inner tube wall and the resulting DNA strands were spooled via the pasture pipet hook.

Stage	Observation	Image
Post heat treatment: Ice bath	The onions sit in the homogenizing solution, but the mixture appears to be heterogenous because it is not uniform, and the layers between the onion and solution can be seen. Temperature measured out to be 18°C	 

Blending and Filtration	After blending, the remaining solution was filled with foam while sitting in the ice bath. Once the solution reached 17°C, the foam subsided a little, leaving behind a little solution and finally when strained through the 4-layer cheesecloth the result was a cloudy liquid as cellular debris remained in the cloth.	
Ethanol Addition	After the addition of ethanol, the solution formed a clear distinction between the lower aqueous extract and the upper alcohol layer. The white and stringy precipitate started to appear in the middle of the two layers.	

DNA Spooling	Translucent and white-like strings were extracted from the solution. The strands appeared mucus like and were slippery as they continued to fall back into the solution. Strands have the visual appearance of a water drop.	
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Post-Lab Questions:

1. Why is SDS an important component in the Homogenizing Solution?

Sodium dodecyl sulfate (SDS) is an important component in the Homogenizing Solution because it is an ionic detergent that disrupts the lipid bilayers of the cellular and nuclear membranes, causing them to break down. It also causes the denaturing of cellular proteins to fully break down biological samples, leaving behind the DNA strands.

2. What do the thickness and viscosity of the extracted DNA suggest about its molecular length?

The higher the thickness and viscosity of the extracted DNA, the more mucus-like the texture is, indicating that the genomic DNA consists of very long and high molecular weight double-stranded polymers. This is because, when released into the solution, these long linear chains get tangled together, creating resistance that leads to a viscous, gel-like mass.

3. Why do RNA molecules typically not spool during this process?

RNA molecules typically do not spool because they are mostly single-stranded and shorter than chromosomal DNA. Since it lacks the length and double-helix structure required to tangle together and wind onto the pipet, the RNA molecules would stay dissolved in the solution.

4. Why is ethanol effective in precipitating DNA from a salt solution?

Ethanol is effective in precipitating DNA from a salt solution because it has a lower dielectric constant than water. This means that when Na^+ ions are present, ethanol reduces the shielding of the negative phosphate groups and allows the sodium ions to

bind and create a neutral charge. Then, once it is neutral, the DNA loses its solubility in water.

5. Predict what would happen to double-strand DNA placed in distilled water. Would it remain stable or denatured?

If the double-stranded DNA were to be placed in distilled water, it would denature by uncoiling and separating into single strands. This is because, in distilled water, there are no Na ions present to neutralize the negative charge, which results in electrostatic repulsion between the strands.

Discussion/Conclusion

The main goal of this experiment was to isolate and visualize genomic DNA from the onion tissue by using a basic biochemical extraction technique. Overall, I would say this experiment was successful because we were able to spool the white- gel like DNA fibers onto the pipette hook at the interface of the aqueous extract and ethanol layers. Through this biochemical extraction, we began by first breaking down the cell structure; since the onion had cell walls of cellulose, we had to dice the onion as well as blend it to break through the outer barrier. But since this alone didn't open up the lipid bilayer, we had to add the homogenizing solution, where the SDS acted as a detergent to solubilize the membrane lipids and essentially release the cellular contents into the solution by breaking down the cell membrane. The heat treatment done by the water bath allowed the cell wall of the onion to soften and speed up the denaturation process. Then we had to transfer the beaker into an ice bath right after to lower the temperature to around 15-20°C because if we left it at a high temperature for too long, then the heat would denature the DNA into single strands. After filtering the cellular debris and plant tissue through the cheesecloth, we were left with an aqueous extract which contained dissolved DNA and salts. DNA is usually soluble in water because the phosphate backbone is negatively charged, and the water molecules form a hydration shell around it. To get the DNA to precipitate, we layered the 95% cold ethanol on top; due to its lower dielectric constant than water, it lowers the polarity of the solvent. This allowed the sodium in the solution to neutralize the negative charges of the DNA phosphate backbone, and once it was neutral, the DNA could no longer dissolve in the polar solution. When we spooled the precipitate onto the hook, the DNA appeared to be a thick, viscous, gel-like substance; this high viscosity reflects the macromolecular structure. The DNA consists of long, continuous double helix chains that tangle together and give the stringy texture. RNA is unable to do this since it is a smaller molecule and doesn't tangle or spool with the lack of length and rigidity.

In conclusion, this lab was a success and demonstrated how specific chemical properties such as SDS disruption of lipids, thermal enzyme inactivation, and salt-ethanol charge neutralization can work together when trying to isolate DNA. This experiment showed me the physical reality of DNA outside of a diagram by proving how its structural length and chemical polarity determine its behavior in a solution.

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