

DNA Extraction

1. Pre-Lab Reading

Chapter 3, Section 5 (3.5) “Nucleic Acids” Biology2e, OpenStax College

2. Purpose and Objectives

The purpose of this lab is to investigate factors affecting the quantity of DNA that can be extracted from plant material.

Upon completion of this investigation, you should be able to:

1. Describe the structure and function of DNA.
2. Describe the structure and function of a detergent.
3. Determine whether different brands of detergent affect the quantity of DNA that can be extracted from a sample of raw wheat germ.

3. Safety Guidelines

- Wear safety goggles
- Handle glassware with care

4. DNA

DNA (deoxyribonucleic acid) is the genetic material found in cells that encodes information responsible for determining the characteristics of living things. In Eukaryotic cells, DNA is found in a membrane-bound compartment called the nucleus. DNA belongs to a class of organic molecules known as **nucleic acids**. There are two types of nucleic acid polymers: DNA

and **RNA (ribonucleic acid)**. Working together, these molecules direct protein synthesis in cells.

DNA is a very large molecule. At physiological pH DNA is negatively charged and therefore quite soluble in water, which is a polar solvent. DNA is not soluble in the less polar solvent ethyl alcohol (ethanol) and will precipitate out of solution when ethanol is added, making the DNA visible.

Extraction of DNA from cells is a common procedure in the molecular biology laboratory. Once scientists have removed DNA from cells it can be used for a variety of purposes such as PCR, sequencing, or even insertion into different cells. Today's experiment uses a relatively simple procedure for extracting DNA from wheat germ. Once the DNA is removed from the wheat cells it will be made visible by precipitating it with ethanol.

Wheat germ contains the embryos of wheat seeds. In the milling process that produces white flour, the wheat germ and wheat bran (protective covering) are removed, leaving behind only the starch. Wheat bran is a good source of dietary fiber and wheat germ contains many nutrients, making whole wheat flour more nutritious than white flour.

The wheat germ we are using is raw and has been purchased at a health food store. It will first be treated with hot tap water to soften the phospholipids found in the cell membranes and nuclear envelopes of the wheat germ cells. The hot water will also denature enzymes (**DNases**) that would cut the DNA into fragments too small to be visible.

The next step is to add shampoo, which contains detergent such as sodium laurel sulfate (or another metal laurel sulfate). Detergents are molecules that have both a nonpolar end and a polar end. They interact with nonpolar membrane lipids and proteins and with water, pulling apart cell and nuclear membranes. Once these membranes are broken apart, the DNA is released from the cell, dissolves in the water/detergent/wheat germ solution and cannot be seen. DNA precipitates out of solution in alcohol, where it can be seen. The alcohol also separates the DNA from other cell components, which are left behind in the water solution.

Different brands of detergent will be used in this experiment. The **question** being asked in this lab is ‘which type of shampoo/detergent used in the extraction process works best for extracting DNA from wheat germ?’

5. Extraction Protocol

Procedure

1. Propose a hypothesis based on the question being addressed in this investigation. Make a prediction based on your hypothesis. Record both your hypothesis and your prediction in your lab book.
2. Students will work individually. Each student will perform one DNA extraction and post data into a class data document.
3. Place 1 gram of wheat germ into a 50 mL centrifuge tube.
4. Measure the mass of one piece of filter paper and record this value in your lab book. Use an analytical balance. **Record this value on the class data sheet as well.**
5. Add 20 mL of hot (50-60°) tap water to your tube and mix constantly for 3 minutes.
6. Using the disposable pipette provided (it has been cut at the 1 mL mark), add 1mL of a detergent to the tube and mix gently every 30 seconds for 5 minutes. The best way to mix is to invert the tube slowly several times, until all of the wheat germ is mixed into the water. Try not to create foam.
7. Allow the test tube to sit for 10 minutes or more until the wheat germ settles to the bottom. Gently pour the water-detergent solution from the tube into a clean test tube, trying not to include any wheat germ. Set aside the tube with wheat germ.
8. Use a pipette or paper towel to remove most of the foam from the top of the solution if needed.

9. Tilt the tube at an angle. SLOWLY pour 14mL of 95% ethyl alcohol down the side the tube with the liquid sample so that it forms a layer on top of the water/wheat germ/detergent solution. Do not mix the two layers together. DNA precipitates out at the water-alcohol interface. Therefore, it is crucial to pour the alcohol VERY SLOWLY so that it forms a layer on top of the water solution. If the alcohol mixes with the water, it will become too dilute, and the DNA will not precipitate out.
10. Let the test tube sit for 15 minutes. White, stringy, DNA will begin to appear where the water and alcohol meet. As the sample sits, more DNA will float from the detergent solution to this layer.
11. Using a glass hook, collect the DNA and place on the filter paper to dry. You can usually get more DNA to precipitate out of solution by using a DNA-collecting tool (in this case a glass hook made from a Pasteur pipette) to gently lift the water solution up into the alcohol. This allows more DNA to come into contact with the alcohol and precipitate out.
12. Each sample will require an overnight drying period. Samples will be weighed for you and data recorded on Canvas. Be sure that you have recorded the mass of the filter paper alone in the class data sheet.
13. Final masses will be recorded and posted on Canvas. Record the final mass for your sample and **calculate and record the mass of DNA** collected in your lab notebook. Record this information for your lab partner's sample as well.
14. Compare your results with the results of your classmates. Data will be provided via Canvas for mass of DNA collected for each shampoo during several lab sections. **Record this Class Data in your lab book** (a printout is fine) as well.

6. Conclusion and Summary

Be sure to turn in the class data table (contains data for several lab sections) and your group data with your conclusions.

1. Calculate the **mean mass** of DNA extracted for each shampoo. Use the class data table posted on Canvas. What do these results show?
2. Do your group data support or not support your hypothesis? Explain. Do the class data support your hypothesis? Explain.
3. Were any results unexpected? How might you account for these unexpected results?
4. What are possible sources of error that could have affected the reliability of your results?
5. Describe limitations in the design of the investigation (for example was data collected in such a way that there was a fair comparison between the different shampoos? Why or why not?).
6. Describe three ways the investigation could be extended. What would you hope to learn?

Acknowledgments

This protocol was developed by Louisa A. Stark, Ph.D. (currently GSLC Education Director) while she was Senior Scientist with the University of Colorado Hughes Undergraduate Biological Sciences Education Initiative, a program funded by the Howard Hughes Medical Institute.

web site

<http://gslc.genetics.utah.edu/basic/wheatgerm/protocol.html>

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