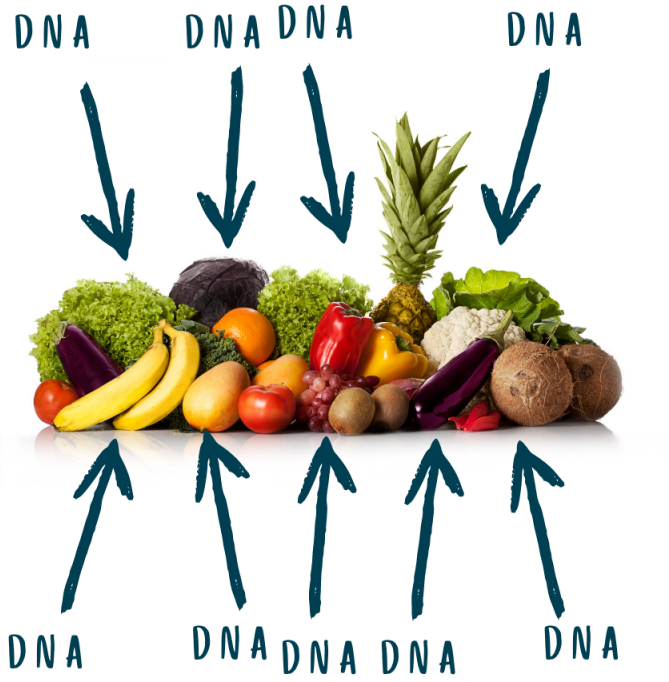
DNA Extraction Toolbox
MiniLab
Student Guide

Cat# M3015

Version 082721



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In this inquiry-based MiniLab, students extract DNA from wheat germ and visualize the concentration of DNA using GelGreen fluorescent dye and the MiniOne Winston blue-light fluorescence reader. Students develop and refine their own DNA extraction protocol by varying the concentrations and proportions of reagents and other variables. Though this hands-on activity, students learn how science progresses through controlled experiments and a bit of trial and error.

This MiniLab contains enough supplies for **10** groups to perform experiments.

Laboratory Safety

1. Wear lab coats, gloves, and eye protection as required by district protocol.
2. Use caution with all electrical equipment such as PCR machines and electrophoresis units.
3. Wash your hands thoroughly after handling biological materials and chemicals.

Introduction

Protocols for DNA extraction can vary from very simple to very complex depending on the tissue and the application. A tough plant stem may need to be physically broken down before DNA can be released from cells, while other cells may need a simple chemical treatment to break down cellular membranes. In this inquiry-based lab you will design experiments to test the effectiveness of various reagents and procedures for extracting DNA from wheat germ, the embryo part of the wheat seed which is commonly used as a nutritional supplement. As an extension activity you can use the principles you have discovered to extract DNA from fruits and vegetables you have in the kitchen.

Reagent Guide

Tween-20: This is a mild detergent that is used to break down the walls of cells. All cells are surrounded by a lipid bilayer membrane. Lipids are similar in chemical properties to grease or fat, and soap helps to dissolve the membrane in the same way that it helps to dissolve grease on pots and pans. Tween-20 is supplied as a 1% solution in water.

Bicarb buffer: This is an alkaline solution (pH ~9.6) which is used to solubilize proteins inside the cell and in the membrane. Cells suspended in alkaline solution are often heated as part of a DNA extraction protocol. This buffer is supplied as a 0.1 M solution.

GelGreen: This is a compound that binds to double-stranded DNA. It is non-fluorescent in solution. When bound to double-stranded DNA, it glows fluorescent green when illuminated with blue light. In this way, it is an indicator that there is double stranded DNA in your solution. Use GelGreen diluted 1:5,000 in water.

Other variables: Variables like temperature or how long the wheat germ is incubated with the extraction solution could affect how much DNA is extracted. Can you think of any other variables that might affect DNA extraction?

Pre lab Questions:

These questions can help you gauge students' prior knowledge before starting the lab activity.

1. Use the internet or your biology textbook to research where DNA is found in eukaryotic cells. Which components of the cell will have to be disrupted to separate the DNA from the rest of the components of the cell?

2. GelGreen is a molecule that is strongly fluorescent when it is bound to double stranded DNA but can't pass through the plasma membrane. This molecule absorbs blue light (the color of the LEDs in the Winston Fluorescence Reader) and emits green light (which is visible through the orange photo hood). How can you use GelGreen and the MiniOne Winston Fluorescence reader to determine whether you have successfully extracted DNA from cells in wheat germ?

3. Look over the Reagent Guide and Other Variables. If you had to pick one and only one from this list to use in your DNA extraction procedure, which one would it be and why?

Part I: Explore DNA Extraction

In your first experiment, you will compare the effectiveness of the reagents used for DNA extraction. Follow the procedure below, paying special attention to step 3. This is where you will modify the procedure in the experiments you design later.

1. Label four microcentrifuge tubes with numbers 1 through 4.
2. To get the same amount of wheat germ in each tube we are going to use the cap of the 0.65 mL tubes to measure the wheat germ. Fill the cap of the tube with wheat germ using your fingers. Flip the tube over the cap to close trap the wheat germ inside the tube.
3. Use a transfer pipette or adjustable volume micropipette to add 200 µL of the solutions below to the indicated tube and shake the tube to mix the liquid with the wheat germ.

Table 1:

Tube #	Reagent(s) in the tube	Rank fluorescence (1 being strongest, 4 weakest)
Tube 1	200 µL water	
Tube 2	200 µL Tween-20 (1%)	
Tube 3	200 µL Bicarb buffer (0.1 M)	
Tube 4	200 µL water +	

For tube 4, add 200 µL water, but change another variable, such as the length of time you allow it to sit before going on to the next step, how long you shake it, or the temperature it's kept at, or another variable of your choosing.

4. Allow the tubes to sit on your bench in a tube rack for one minute.
5. Use a transfer pipette or adjustable volume micropipette to remove 100 µL of the liquid above the wheat germ, called as the supernatant. Be careful not to disturb the wheat germ at the bottom. Add the supernatant to a clean microcentrifuge tube labeled with the same numbers as used above.
6. Use a transfer pipette or adjustable volume micropipette to add 100 µL of the GelGreen solution to each tube with the supernatant. Cap the tubes and invert a few times to mix.
7. Place the tube in the Winston Fluorescence Reader and put the photo hood on top. Use your cell phone camera to document the fluorescence in the tubes.
8. Visually compare the brightness of the fluorescence in the tubes. Which ones are the brightest? Which are the dimmest? What does this tell you about the concentration of DNA in the solution? Which reagents or variables do you think had the biggest effect on the quantity of DNA extracted? Record your answers in Table 1.

Part II: Develop your protocol

Estimated Time: 20 min

In Part 1, you likely identified one or more factors that affect the amount of DNA that you can extract from wheat germ. Pick one reagent or variable to investigate in more detail. If you are interested in one of the reagents you could try varying the concentration by doing a serial dilution of the stock solution. If you are interested in heat or time you could systematically vary the temperature or the length of time you incubate the wheat germ in the solution you've chosen to add.

Again, you will test four conditions in four tubes. Look over the list of reagents and variables above and consider which you would like to compare in a DNA extraction experiment. Design your experiment in the space below, then follow the general procedure above to extract DNA from wheat germ and compare DNA concentration between tubes. Record what you are going to add to each of the tubes and any other relevant variables (e.g. incubation time, or temperature).

Which variables will you keep the same between tubes?

Which variable will you change between tubes?

What will be your control treatment? (That is, which tube will you compare the other tubes to?)

Use the space below to diagram your experiment:

How does changing the variable affect the concentration of DNA that you extracted?

Which level of the variable would you choose for optimal DNA extraction concentration?

Part III: Refine your approach

Estimated Time: 20 min

In Parts 1 and 2 you identified variables that affect DNA extraction and may have gotten closer to identifying optimal conditions for extracting DNA from wheat germ. In Part 3, your task is to resolve one remaining question you have. Perhaps you found that longer incubation time yields higher concentrations of DNA in water. Here you could test whether longer incubation time also gives better extraction in a Tween-20 solution. Maybe you determined that heating the sample gives better DNA extraction in water. Here you could test whether this holds true when bicarb buffer is used.

Again, use the questions below to plan your experiment, then follow the general procedure to extract DNA from wheat germ and compare DNA concentration between tubes.

Which variables will you keep the same between tubes?

Which variable will you change between tubes?

What will be your control treatment?

Use the space below to diagram your experiment:

Based on your results in Parts 1 - 3, what is the best protocol for extracting DNA from wheat germ? Describe the solution that you would add to the tube with the wheat germ and any other treatment you would apply to the tube.

Part IV: Optional extension activity

In this optional activity, you will use the principles you learned in Parts 1-3 to extract DNA from fruits or vegetables in your house. This experiment will be slightly different from the wheat germ extractions because instead of visualizing DNA concentration in solution with fluorescent GelGreen, we will be precipitating and "spooling" the DNA. We will demonstrate DNA extraction using inexpensive materials you can find around the house.

Note: if you are using dry beans you will have to soak them to rehydrate. You can soak them in room temperature water overnight or in hot water for a couple hours. You will know they are rehydrated when you can easily peel the skins off. Skins should be removed from the beans before doing the extraction.

1. Prepare 100 mL of your "extraction solution". Here are some tips for how to take the principles you have learned in Parts 1-3 and apply them to this new experiment:

    - In place of Tween-20, you can mix water with liquid detergent.
    - You can make your own bicarb buffer by mixing baking soda with water.
    - To precipitate and spool the DNA we will have to add a small amount of salt to the mixture. Salt neutralizes the negative charges on the DNA backbone and allows the DNA strands to cluster together and precipitate.
    - Choose a fruit or vegetable to extract DNA from. We have found that strawberries, kiwis, onions, and dry beans work well.

2. Place the fruit or vegetable you have chosen in a plastic ziplock bag. Use one strawberry with the stem and leaves removed, a couple slices of kiwi or the equivalent amount of another fruit or fresh vegetable. If using beans, remove the skins, place about 10 in the plastic bag and smash with a rolling pin or the flat bottom of a cup.

3. Pour about 25 mL of extraction solution into the bag. *Gently* squash the fruit or vegetable with your hands until all large chunks are gone. If you are using detergent, be careful to create as few bubbles as possible.

4. Position the mesh strainer over a beaker or clear plastic or glass cup and pour the contents of the bag into the strainer to separate the solids from the liquids. When all of the liquid has drained through, set the strainer aside.
5. Gently pour 10 mL isopropyl alcohol down the side of the cup or beaker so that it forms a layer floating on top of extraction liquid.
6. Hold the cup or beaker at eye level and examine the interface between the two liquids. You should see a white stringy substance. This is the DNA! Carefully wind the DNA around a chopstick or wooden stir stick and lift out of the liquid. This is called "spooling" the DNA. If desired, you can save the DNA in a microcentrifuge tube.
7. Optional: repeat this DNA extraction experiment changing a single variable as we did with the wheat germ experiments. How does this variable affect the quantity of DNA you can collect? Try varying the concentration of detergent or salt, the length of time you spend squishing the fruit or vegetable with the extraction solution, or the temperature that you heat the bag.

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