

DNA ISOLATION

Equipment & Supply List

<u>Sent Out</u>	<u>Returned</u>	
<input type="checkbox"/>	<input type="checkbox"/>	1 ice container (cooler)
<input type="checkbox"/>	<input type="checkbox"/>	1 hot water bath
<input type="checkbox"/>	<input type="checkbox"/>	6 conical 50 ml tubes containing raw wheat germ
<input type="checkbox"/>	<input type="checkbox"/>	1 container with extra wheat germ (if necessary)
<input type="checkbox"/>	<input type="checkbox"/>	6 conical 50 ml tubes containing 91% ethanol or isopropanol
<input type="checkbox"/>	<input type="checkbox"/>	6 conical 50 ml tubes with detergent
<input type="checkbox"/>	<input type="checkbox"/>	1 container with extra detergent (if necessary)
<input type="checkbox"/>	<input type="checkbox"/>	6 square ice bath containers
<input type="checkbox"/>	<input type="checkbox"/>	6 rectangular colored test tube racks for 50 mL conical tubes
<input type="checkbox"/>	<input type="checkbox"/>	plastic applicators in bag (for spooling DNA)
<input type="checkbox"/>	<input type="checkbox"/>	1 large plastic beaker containing 3 ml graduated plastic pipettes
<input type="checkbox"/>	<input type="checkbox"/>	1 glass container with 50% ethanol/isopropanol for DNA storage
<input type="checkbox"/>	<input type="checkbox"/>	50mL conical tubes with caps for DNA isolation
<input type="checkbox"/>	<input type="checkbox"/>	15mL graduated tubes for further DNA isolation
<input type="checkbox"/>	<input type="checkbox"/>	smaller clear test tubes for DNA storage
<input type="checkbox"/>	<input type="checkbox"/>	small plastic caps for small test tubes
<input type="checkbox"/>	<input type="checkbox"/>	CCA Science DVD
<input type="checkbox"/>	<input type="checkbox"/>	Microcentrifuge tubes option to save DNA in (necklaces)
<input type="checkbox"/>	<input type="checkbox"/>	3-ring notebook with protocols and other supplementary materials

***Note: You will need to supply:

- crushed ice for ice baths at each lab table

DNA ISOLATION

Materials and Supplies Needed

Students work in lab pairs

- raw wheat germ (approx. 1 teaspoon per lab pair)
- 50° C tap water (50 ml per lab pair)
- dishwashing detergent solution (approx. 2 ml per lab pair)
- plastic disposable (2 ml) pipettes (4 per table of 2 lab pairs)
- round, plastic applicators (1 per lab pair)
- 91% (ice cold) ethanol or isopropanol (5 ml per lab pair)
- Ice containers for isopropanol
- 50mL conical tubes with screw-cap lids
- 15mL graduated tubes
- hot water bath

Optional:

- * small capped tubes containing 50% ethanol or isopropanol for DNA storage (1 per student)
- * OR microcentrifuge tubes with string for DNA storage (as necklaces)

DNA ISOLATION

Pre-Lab Preparations

***Note: Ethanol/Isopropanol MUST be stored in a freezer prior to and after use.**

1. Remove 91% ethanol or isopropanol from freezer and place immediately in an ice bath to chill. It must remain on ice for the entire lab in order to work properly.

2. Heat water to 50° C in a water bath or let tap water run to 50° C.

3. Set out supplies at each table of two lab pairs. Each lab table should have:

- small jar or tube with raw wheat germ
- tube of dishwashing detergent solution with pipette
- 2 disposable plastic pipettes
- 2 wooden sticks, glass stirring rods, or “hooked” pipettes
- ice bath for test tubes of ethanol
- 50ml conical tubes with screw-cap lids
- 15ml graduated tubes

* small screw-cap test tubes with 50% ethanol (optional)

* OR microcentrifuge tubes with string to save DNA (necklaces)

DNA ISOLATION

Procedures

For each lab pair of students

1. Place approximately 1 teaspoon of raw wheat germ (just enough to cover the entire tip) in a 50ml conical tube. You should not be able to see the bottom of the tube.
2. Add 20mL of hot (50° C) water from the tap or water bath to the wheat germ using a second conical tube.
3. Cap the tube with the wheat germ and water tightly and mix thoroughly by shaking constantly and vigorously. The solution must be agitated for 3 minutes.
4. Add 20 drops of liquid dish soap using a plastic disposable pipette and dropping it down the side of the conical tube.
5. Mix gently with a plastic applicator every minute for 5 minutes.

****You may want to remove the aqueous (liquid) top portion of your wheat germ and place it into a smaller, 15mL graduated tube before moving onto step 6 to ensure a greater yield of DNA.****

6. Slowly add 1-2 ml of **ice cold** ethanol or isopropanol by dripping it down the side of the wheat germ conical tube with a plastic pipette. This step yields a bilayer between the alcohol and the water. Watch for the precipitation of DNA (white cloudy substance) to appear in the ethanol/isopropanol. Most will appear quickly but some will precipitate over the next few minutes. You may wish to add a second (and perhaps a third) 1 ml of ethanol or isopropanol to precipitate even more DNA.

7. Let the tube sit for a few minutes.

10. Use the end of a thin plastic applicator to slowly wind the DNA in the test tube until it appears that most of the DNA is wound into a mass.

11. To remove the DNA from the test tube, carefully tilt the test tube and press the stick or rod against the side of the tube and drag it towards the top. The DNA may fall off if you just raise the stick or rod directly up out of the ethanol. Alternatively, you may pour the DNA & ethanol solution onto filter paper or paper towel in a shallow dish and allow to air dry.

The following step is optional:

12. To save the DNA (for later use or to take home to show others) place it in small capped test tubes or microcentrifuge tubes containing 50% ethanol or isopropanol.