WHEAT GERM DNA EXTRACTION

Introduction:

This is a DNA extraction and isolation activity using common household chemicals. With dish soap, meat tenderizer, baking soda, and alcohol, students will isolate DNA from raw wheat germ.

Materials Needed:

Raw (untoasted) wheat germ, 2g
Liquid detergent (Palmolive®, Dawn®) 3mL
Meat tenderizer (unseasoned original), 2g
Cold Alcohol, 95% (ethyl or isopropyl), 20mL, -20°C store in freezer
Water bath at 55°C
1.0 M Sodium bicarbonate, $NaHCO_3$ (baking soda)
Paper clip (giant- sized)
Tap water
Thermometer
Beaker or cup (200-mL/8 oz.)
Balance/weight boat
Graduated cylinder, 100 mL, 10 mL
Ice bath
Serological pipet, 10-mL/pumps
Scoopula

Pre-lab:

The alcohol should be ice cold, approx. 0°C or less, leave in freezer until ready to use. Prepare a 1 molar sodium bicarbonate solution by dissolving 8.4g of $NaHCO_3$ in 100 mL of tap, or distilled water. It is essential that the wheat germ be raw—toasted wheat germ will not work Raw wheat germ can be fond in health food stores and in some grocery stores.

Straighten the paper clip and form a small hook at one end. Roughen the hook portion with a file—a roughened surface enhances adhesion of the DNA strands, and facilitates spooling.

Procedure:

1. Measure 45 mL of tap water into a beaker or cup and place it in a warm water bath. Allow it a few minutes to warm to at least 50°C. The optimal temperature for the procedure is 55°C—do not allow the temperature to exceed 60°C.
2. Sprinkle the wheat germ into the beaker and gently stir in 3mL of detergent. Allow this mixture to incubate in the 50°–55°C water bath for 10 minutes.
3. After 10 minutes, gently stir in 2g of the meat tenderizer and 5mL of the 1 *M* sodium bicarbonate solution. Incubate this mixture at 50°–55°C for an additional 10 minutes.
4. Transfer the beaker containing the wheat germ mixture to an ice bath for a few minutes to quickly cool it to approximately 25°C. Swirl gently during this period.
5. Place the beaker on the table. Using the serological pipet, carefully layer the ice-cold alcohol over the wheat germ solution in the beaker. Allow the alcohol to slowly flow from the pipet with the pipet tip held against the inside surface of the beaker just above the liquid level.
6. There will be a visible interface between the alcohol layer and the wheat germ mixture layer. A cloudy looking fibrous white precipitate should be evident at the interface. This is DNA. Use the paper clip hook, immersed to the depth of the interface, to spool up the DNA fibers. Use a slow, twirling motion to avoid breaking and separating the strands. It will appear as slimy white "snot-like".

7. Place about a BB size bit of the DNA in a micro centrifuge tube with about 0.5mL 95% ethanol.
8. Label and store in freezer if future experiments are planned.

Discussion:

Wheat germ is the embryo (sprouting) section of the wheat kernel; the remainder being the endosperm (storage). The germ is extremely rich in vitamins and nutrients, and for the purposes of this experiment, an excellent source of DNA. The steps in this procedure can teach us a great deal about the properties of cells, cell membranes, and of deoxyribonucleic acid (DNA) itself. So why did we do what we did?

Detergents solubilize and break down the lipids and proteins that form the primary cell membrane and disrupt the bonds that hold the membrane together. The cell contents, including the nucleus, are thus released for further treatment. Heat is applied to assist in softening the cell membranes and enhancing the action of the detergent. The heat also denatures enzymes that might otherwise damage the DNA. You were also cautioned to keep the temperature below 60°C—because higher temperatures denature the DNA and make spooling impossible. Eventually, even at 55°C, the DNA would break down. For this reason, the incubation period is limited to 15-20 minutes, and the mixture must be cooled quickly once the incubation is complete.

The sodium bicarbonate solution is added to maintain a near-neutral pH—at which the DNA is most stable and at which the enzyme present in the meat tenderizer is most effective. The meat tenderizer contains the proteolytic (protein breaking) enzyme papain—naturally present in papaya, pineapple, and other fruits. The papain completes the breakdown of the nuclear membrane at which point the DNA is freely in solution.

The final step requires the cold alcohol. The solubilized DNA contacts the alcohol where the two liquid layers meet. The alcohol dehydrates and precipitates the DNA, as DNA is insoluble in the alcohol (especially cold alcohol). If the procedure is carried out properly, fine, long strands of DNA will form at the interface—and can be readily spooled onto the paper clip.

Safety Precautions:

Ethyl and isopropyl alcohol, 95%, are flammable and dangerous fire risks; keep from flame and sources of ignition. Both alcohols are also toxic by ingestion. Chemical splash goggles are advised whenever heat and glassware are used.

Disposal:

The resulting mixtures can be rinsed down the drain.

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