Isolating DNA from Fruits

Introduction

In 1962, James Watson (1928–), Francis Crick (1916–2004), and Maurice Wilkins (1916–2004) received the Nobel Prize for their determination of the structure of deoxyribonucleic acid (DNA). In the years since the structure of DNA was first unraveled, it has become the most significant biological topic of the century. Understanding the structure of DNA helps to explain many life processes and genetic differences between organisms. The process of DNA extraction is of primary importance in many fields of biotechnology. It is critical for genetic research, DNA fingerprinting, and creating recombinant organisms to produce beneficial products in the field of medicine.

Concepts

- DNA spooling/isolation
- Cell lysis
- Solubility

Background

Plant cells are often *polyploid*, meaning that each cell has more than two copies of the same chromosome. Polyploid cells therefore contain an abundance of DNA, making polyploid organisms practical for extracting and isolating DNA. In order to isolate DNA from fruit sources, the cell walls, cell membrane, and nuclear membranes must be disintegrated or *lysed*, the enzymes that naturally digest DNA must be disabled, and the DNA must be precipitated out of the resulting solution.

In this activity, lysing the cell wall of a piece of fruit is accomplished by quickly blending or smashing the fruit. Salt is added to the filtered fruit solution to coalesce (combine) the DNA strands that have been freed from the nucleus. Detergent is added to the mixture to break apart and emulsify the lipids and proteins that make up the cell and nuclear membranes. Sodium dodecyl sulfate is an emulsifier used in detergents. Finally, the DNA-destroying enzyme DNase is disabled by adding ethylenediaminetetracetic acid (EDTA). DNA is soluble in water and insoluble in ethyl alcohol. Adding ethyl alcohol to the top of the chemically treated fruit mixture dehydrates and precipitates the DNA from the solution. The DNA precipitates at the water/alcohol interface and can be collected.

Materials (for each student)

Ethyl alcohol, 95% denatured, CH_3CH_2OH, 12 mL
Ethylenediaminetetracetic acid solution (EDTA), 0.1 M, 1 mL
Sodium chloride solution, NaCl, 8.0%, 1 mL
Sodium dodecyl sulfate solution (SDS), $CH_3(CH_2)_{11}OSO_3Na$, 10%, 1 mL
Water, deionized or distilled, 40 mL
Banana, strawberry or kiwi
Beakers, 50-mL, 2
Cheesecloth
Graduated cylinders, 25-mL, 2
Ice-bath
Inoculating loop
Pipets, graduated, disposable, 3
Resealable bag
Test tube, 5-mL

Safety Precautions

Ethyl alcohol is flammable and a dangerous fire risk— keep away from flames and other sources of ignition. Sodium dodecyl sulfate solutions may be irritating to skin. Any food-grade items that have been brought into the lab are considered laboratory chemicals and are for lab use only. Do not taste or ingest any food in the lab and do not remove any remaining food items. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Wash hands thoroughly with soap and water before leaving the laboratory. Follow all laboratory safety guidelines. Please review current Safety Data Sheets for additional safety, handling, and disposal information.

Publication No. 10805CA
050217

Preparation

1. Prepare an 8.0% sodium chloride solution: Dissolve 8.0 g of sodium chloride in about 70 mL of deionized water. Dilute to a final volume of 100 mL with deionized water. Place the solution in a labeled bottle.
2. Prepare a 10% sodium dodecyl sulfate solution (SDS): Dissolve 10.0 g of sodium dodecyl sulfate in about 70 mL of deionized water. Dilute to a final volume of 100 mL with deionized water. Place the solution in a labeled bottle.
3. The 95% ethyl alcohol should be ice cold (about 0 °C) when used. Place it in an ice bath before use.

Procedure

1. Pour 40 mL of deionized water into a clean, resealable bag.
2. Place a one-inch section of banana, kiwi or a strawberry into the resealable bag.
3. Smash the fruit in the resealable bag until the fruit mixture has a thick liquid consistency.
4. Pour the fruit mixture through four layers of cheesecloth which is layered on the top of a clean 50-mL beaker. *Note:* Do not squeeze the cheesecloth.
5. Use a clean, graduated cylinder to transfer 10 mL of the filtered fruit solution into a clean beaker.
6. Use a clean, graduated pipet to add 1 mL of the 8% sodium chloride solution to the beaker. Mix well.
7. Use a clean, graduated pipet to add 1 mL of the 10% SDS solution to the beaker. Mix well.
8. Use a clean, graduated pipet to add 1 mL of the 0.1 M EDTA to the beaker.
9. Holding the beaker at a slight angle, use a clean, graduated cylinder to carefully transfer 10 mL of ice-cold 95% ethyl alcohol down the side of the beaker so that the ethyl alcohol forms a layer on top of the solution in the beaker (see Figure 1).
10. Carefully place the beaker back on the tabletop, making sure the two layers do not mix.
11. Allow the beaker to sit for a few minutes and observe the DNA precipitating out of the water solution at the interface between the cold ethyl alcohol and the aqueous layers.
12. Add 1 mL of cold 95% ethyl alcohol to a small test tube.

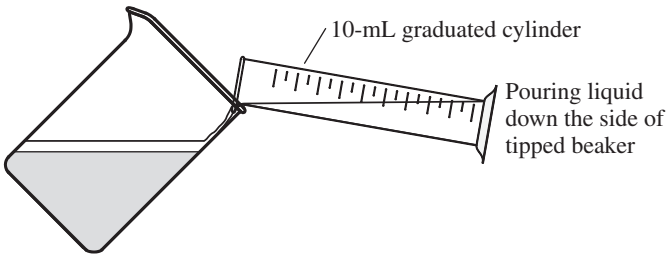

Figure 1.

13. Gently place the inoculating loop into the interface containing the DNA. Collect the DNA by turning the loop in one direction —the DNA will "wind" itself onto the loop.
14. Carefully remove the loop and DNA from the beaker and transfer the DNA to the small test tube containing the ethyl alcohol. Observe the DNA strands floating in the alcohol.

Disposal

It is recommended that you consult your local school board and/or municipal regulations for proper disposal methods that may apply before proceeding.

Tips

- Sodium dodecyl sulfate is also known as dodecyl sulfate, sodium salt, sodium lauryl sulfate, and SDS.
- A test tube, culture tube or centrifuge tube may be substituted for the 50-mL beaker.
- Fresh or dried banana, fresh strawberries, fresh kiwi, fresh onions, raw wheat germ, and liver all also give good yields of DNA. Many angiosperms are polyploids. For example, bananas have three copies of each chromosome (triploid), and strawberries have eight copies of each chromosome (octoploid).
- The procedure described above was optimized using seven dried banana chips soaked overnight in 100 mL of deionized water.
- A coffee filter is a good substitute for cheesecloth when filtering the fruit solution.

Materials for *Isolating DNA from Fruits* are available from Flinn Scientific Canada, Inc.

Catalogue No.	Description
EJ0009	Ethyl Alcohol, 95%, 500 mL
SJ0064	Sodium Chloride (NaCl), Laboratory Grade, 2 kg
DJ0024	Dodecyl Sulfate, Sodium Salt (SDS), 100 g

Consult www.flinnsci.ca or your *Flinn Scientific Canada Catalogue/Reference Manual* for current prices.