Liver and Onions:

DNA Extraction from Animal and Plant Tissues

by Karen J. Nordell, Anne-Marie L. Jackelen, S. Michael Condren, George C. Lisensky, and Arthur B. Ellis*

Integrating the Activity into Your Curriculum

This activity, which allows students to extract DNA from plant and animal cells (1), serves as a spectacular example of the complexity of biochemical structure and function and fits well with a discussion of nucleic acids, hydrogen bonding, genetic coding, and heredity. DNA extraction can also be used in conjunction with a discussion of polymers and their properties. This activity can be used to complement a diffraction experiment illustrating how the double helix structure of DNA was determined (2).

About This Activity

This activity should be done in the chemistry laboratory with supervision by the instructor. It is not a take-home activity. Students should wear splash-proof goggles.

Materials: raw beef liver, onions (and/or other vegetables or fruits such as banana and kiwi), knife, distilled water, sodium chloride, sodium bicarbonate, liquid detergent (Ivory recommended), 3 M sodium acetate solution (prepared by the instructor), ethanol, balance, ice-water bath, blender, 100-mL Erlenmeyer flasks, 15-mL beakers, 10-mL and 100-mL graduated cylinders, glass rod, 150×13 -mm test tubes, centrifuge (if a centrifuge is not available, cheesecloth or a Buchner funnel with no filter paper), dropper

In order to extract DNA, the cell membrane is broken mechanically, allowing the DNA and other cellular materials such as proteins to spill into solution. The cell walls of plant tissue are more structurally rigid than the cell membranes of animal tissue. For this reason, the extraction procedure described in this activity results in better yields with beef liver than with onion or banana. It is instructive, however, to extract DNA from a variety of tissues to illustrate the ubiquity of the DNA molecule.

Once the cell membranes have been broken, the cell contents spill into the solution, which is saline buffered (pH 8) to stabilize the DNA molecules. The buffer also contains a detergent that has two purposes: to break down the cell walls and membranes further, and to denature enzymes that might destroy the DNA (*3*). Ivory dishwashing detergent is recommended but laundry detergents and shampoos may also work. To save time, the buffer can be prepared and chilled in advance. The DNA molecules are soluble in aqueous saline solutions, but will precipitate when cold ethanol is added in the final step of the extraction procedure. If the DNA has not been too fragmented, students may be able to spool many long parallel strands of DNA molecules onto a glass pipet.

Standard DNA characterization methods include a comparison of the UV absorption at 260 to 280 nm, enhanced fluorescence from intercalating agents, gel electrophoresis, and a chemical assay using diphenylamine ($3-\theta$). The presence of DNA in the fibrous precipitate obtained in this activity has been confirmed using fluorescence and chemical assay methods; the DNA precipitated in this experiment is not sufficiently pure to be characterized by the UV absorption method.

More Information

- 1. Carlson, S. Scientific American. 1998, 279(3), 96-97.
- Lucas, A. A.; Lambin, Ph.; Mairesse, R.; Mathot, M. J. Chem. Educ. 1999, 76, 378–383. A DNA Optical Transform Kit by the authors of this activity will be available from the Institute for Chemical Education, 1101 University Ave., Madison, WI 53706-1396; phone 800/991-5534.
- 3. Boyer, R. F. Modern Experimental Biochemistry; Benjamin/Cummings: Menlo Park, CA 1986, pp 425-434.
- 4. In general DNA intercalators are very hazardous chemicals. They need to be manipulated very carefully in low concentrations and safely disposed of by chemical decomposition, as described in their Materials Safety Data Sheets. This activity focuses on the qualitative observation of DNA by following simple and safe procedures.
- 5. Burton, K. Biochem. J. 1956, 62, 315.
- Pure solutions of DNA can be purchased as a standard against which students can compare their DNA samples by observation or by using one of these characterization techniques. A DNA Spooling Educational Kit is available from Sigma (phone 800/325-3010, prod. no. D8666) for about \$20.

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JCE Classroom Activity: #15

Student Side

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The deoxyribonucleic acid (DNA) molecule is composed of two complementary polymer strands wrapped around each other and held together by hydrogen bonding. The monomer, or building block, of this extraordinary polymer consists of a phosphate, a simple sugar, and one of four nitrogen-containing bases. The phosphate and sugar groups comprise the backbone of each helix. Each monomer contains one of the four bases: thymine, adenine, cytosine, and guanine. The base pairs thymine– adenine and cytosine–guanine are complementary and hold the strands together with two and three hydrogen bonds, respectively. The polymeric nature of DNA allows it to carry the instructions for the function and replication of a complete organism. So detailed are these instructions that some DNA molecules contain as many as several hundred million base pairs.



In this activity, you will extract DNA from the cells of common foods. In order to do this, the cell membrane is broken by mechanically crushing the tissue and by adding a buffered detergent solution. Once the cell

membranes are broken, the DNA and other cellular materials such as proteins spill out of the cells. DNA molecules are fragile and can break easily, so be gentle. DNA is soluble in slightly basic salt solutions and precipitates in ethanol.

Try This

Wear splash-proof goggles at all times. Do not eat, drink, or taste any of the materials used in the activity.

- _1. Solution Preparation. Chill a flask containing 50 mL of ethanol in an ice-water bath. Prepare a carbonate buffer solution by dissolving 0.75 g of NaCl, 2.5 g of NaHCO₃, and 1 mL of liquid detergent in 60 mL of distilled water. Chill the solution in an ice-water bath. Obtain about 1 mL of an aqueous 3 M sodium acetate solution.
- 2. Extraction of DNA from Liver. Place 5 cm³ of raw beef liver (fresh or frozen) and about 30 mL of distilled water in a blender. Purée the liver by pulsing the blades on and off several times for a total blending time of 20–30 seconds. The resulting mixture should look like a chunky red milkshake. Pour the puréed mixture and an equal volume of the chilled buffer solution into a small beaker and stir with a glass rod for two minutes. Transfer about 10–15 mL of the mixture to a test tube (use the size that fits your centrifuge) and centrifuge for three minutes. The denser materials will spin to the bottom of the tube, leaving the DNA solution on top. If a centrifuge is not available, separate the DNA solution from the other cellular materials by straining the mixture with a cheesecloth or a Buchner funnel used without filter paper.
- <u>__3</u>. **Precipitation of DNA.** Transfer about 5 mL from the top of the mixture from the centrifuge tube (or the filtrate) into a clean 150×13 mm test tube, add 10 drops of aqueous 3 M sodium acetate solution, and gently stir the solution. Carefully pour 10 mL of **ice-cold ethanol** down the side of the test tube so that two layers form. The DNA will appear at the interface between the layers. If long polymer fibers are obtained, they can be spooled around a glass rod or disposable pipet like spaghetti around a fork.
- ____4. **Extraction of DNA from Onions**. Repeat the procedure substituting onion for the liver. You may need to increase the blending time. If time permits, try the same procedure with other vegetables and fruits such as banana and kiwi.

Questions

- __1. Would you expect the same results from different cellular materials? Do your experimental results confirm your prediction?
- __2. Would you expect the same results for different amounts of blending?
- _3. DNA molecules can be very long, but their width is only a relatively small number of atoms. Can you see a single molecule of DNA?

Information from the World Wide

Web (accessed Jan 1999)

1. DNA http://mrsec.wisc.edu/EDETC/DNA.htm

one helical turn = 3.4 nm Sugar-phosphate backbone Base Hydrogen bonds

The Structure of DNA

Extracting DNA from a cell's nucleus (© Copyright 1998 Genentech, Inc., used with permission)

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