To close the yellow note, click once to select it and then click the box in the upper left corner.
To open the note, double click (Mac OS) or right click (Windows) on the note icon.

#22 DNA on a Stick

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INTRODUCTION

Description

What is the chemical basis of life? What kind of chemical is DNA? The first step in the genetic manipulations involved in biotechnology is the isolation of DNA. This project describes one method of chromosomal DNA isolation with minimum breaks. There are several basic steps in DNA extraction. The cell must first be lysed (broken open) to release the nucleus. The nucleus must also be opened to release the DNA. At this point the DNA must be protected from enzymes that will degrade it, causing shearing. Once the DNA is released, it must be precipitated in alcohol.

Student Audience

This hands-on activity is conducted by Biology I honors students with the help of the instructor.

Goals

The concept of DNA structure is a challenging one for teachers to teach and for students to learn. Unfortunately, it is difficult to envision actual DNA. This experiment provides a “hands-on” activity and opportunity for students to get visual evidence of the physical nature of DNA and the process of DNA purification. By the end of this lab activity, students will:

- gain experience using lab equipment such as a centrifuge and pipettes,
- get practice using the microscope properly, and
- gain practice using various measuring techniques.

Since following directions is critical to this procedure, students will achieve greater success if all steps are followed.

Recommended Placement in the Curriculum

The DNA isolation follows detailed cell study and protein synthesis. Assessment reveals a much greater understanding of related concepts following this lab.

STUDENT HANDOUT

DNA Extraction from Calf Thymus

Purpose

The purpose of this experiment is to extract high molecular weight chromosomal DNA from a buffer solution of calf thymus. Spooling techniques help students visualize the genetic material separated from within the nuclei by ethanol precipitation.

Scenario

Researchers report that chromosomes of haploid mammalian body cells can each contain DNA molecules greater than 50 million base pairs. To be able to comprehend real world applications such as cloning, one needs to understand that DNA serves as the molecular basis of heredity. It is also important to relate DNA structure to replication and protein synthesis.

Safety, Handling, and Disposal

- Use caution when operating the centrifuge.
- Dispose of used reagents according to local ordinances.
- Wear latex gloves and goggles during the procedure.
- The laboratory surfaces should be very clean during all procedures used in this activity.
- Use thoroughly clean instruments and glassware. Rinse all equipment with isopropyl alcohol or acetone.
- Ethanol is highly flammable; use caution.

Materials

(for group of 30 students)

10 grams fresh or frozen calf thymus	sharp scalpel	200 mL cold prep buffer
kitchen blender	6 solid glass rods	ice
cheesecloth or centrifuge	6 centrifuge tubes	glass pipettes
6 50-mL graduated cylinders	timers	6 50-mL beakers
ice cold ethanol	2 M NaCl solution	SDS solution
EDTA solution	6 small test tubes	latex gloves

Procedure

(For maximum time efficiency, the instructor could complete steps a-c before students arrive.)

I. Homogenizing the Tissue (For every 30 students)

a. Carefully chop 10 g fresh or frozen calf thymus into tiny pieces (without thawing). Thymus (often called sweet bread) needs to be ordered from a butcher shop and should be fresh.

b. Combine it with 200 mL of prep buffer. Kitchen blenders are quite acceptable if one is careful not to overdo the blending. Discard any obvious bits of connective tissue.

c. Place the chunks into the chilled prep buffer on ice. (It is a good idea to wear latex gloves during this procedure, and use thoroughly clean instruments and glassware, preferably rinsed with alcohol or acetone and dried). Homogenize the tissue on ice if possible. If you are using a kitchen blender, stop frequently and put the pitcher part on ice for a few minutes. After 3 minutes of blending, there should be a completely opaque, foamy suspension of nuclei on top.

d. Either strain the homogenate (resulting mixture) through about four layers of cheesecloth or divide equally into six centrifuge tubes and spin at low speed in a table-top centrifuge for 5 minutes to sediment the debris.

e. Use the supernatant (upper layer that contains the DNA) in the next spin. Spin down the nuclei (lower layer) at nearly top speed in the centrifuge. Four minutes of spin should give a substantial pellet of a fairly whitish color. This is the nuclear pellet.

f. Pour off the supernatant, holding the pellet on the upper side of the tube, and resuspend the pellet in fresh prep buffer.

g. Replace the full amount of prep buffer, and mix gently. (One should stop at this point for a microscopic examination of the material; stain first with methylene blue, and use a cover slip).

II. Lysing the Nuclei

a. Put about 40 drops of the nuclear suspension (well mixed) in a small test tube.

b. Add 20 drops of EDTA solution, mix gently, and let stand for 5 minutes. (Removes magnesium and calcium and begins to weaken the nuclear membrane, while it inhibits DNAses to prevent DNA degradation).

c. Add 6 drops of SDS solution, mix very gently, and let stand 1 minute. (SDS is a mild organic detergent which dissolves the lipids in the nuclear membrane while it deproteinizes the DNA and removes the histones).

Note: From now on, do all your pipetting very slowly, and avoid any harsh or sudden movement of the test tube. The chromatin is rather fragile, and as you perform the next few steps it will become more so.

III. Salting Off the Protein

This step provides lots of ions to interfere with the ionic bonding between protein and DNA:

a. **Slowly** add 6 drops of 2 M NaCl to your tube, pausing after every drop, gently mixing. (Mixing should be a slow, circular movement of a clean pipette tip.)

b. **Very gently** pour the contents of the tube into a 50-mL beaker or test tube.

Your protein, now removed from the DNA, will change the appearance of the contents of the tube. (It should begin to appear much more viscous and cloudy than previously.)

IV. Separation of the DNA from the Protein Solution

This is a simple step that precipitates one substance and not the other. Alcohol precipitation is one of the simplest and most effective methods. The object is to layer ice cold ethanol or isopropanol on top of the solution in the tube, rather than mixing it in. Ethanol will mix with water; you are simply taking advantage of its lower density to keep the two separated.

a. First, using a clean pipette and some 100% (absolute) ethanol (isopropanol works quite well), fill the pipette with about the same volume of alcohol as the “mixture.”

b. Hold the small beaker of salted chromatin so that you see it against a dark background, and brace your arms firmly on the table.

c. Making absolutely sure your hands are steady, very carefully trickle the ethanol down the side of a clean glass stirring rod, slowly at first until a layer is visible on top of the chromatin solution, then faster; just be careful not to agitate the layer.

d. At this point, you should see a whitish precipitate forming. Rotating the glass rod, use a gentle, circular motion as if you were trying to scoop something off the walls of the beaker to begin the spooling process. Dip slightly down into the DNA layer and then up into the alcohol, and repeat.

You should soon see strands of DNA winding out on your rod; if not, dip a little deeper and pull up a little higher until you do. The DNA precipitates from solution at the mixing zone and collects on the rod. Once the spooling starts, you can be a little less gentle. Keep on spooling until you have a large clump of DNA on your probe. Squeeze out the ethanol from the glob of DNA on the rod by pressing the product against the side of the beaker.

Note: If the DNA is of very high purity, it will be pure white or, to be more accurate, somewhat iridescent, like an opal. Take time to examine it closely in different lighting conditions.

V. To Save the DNA Produced

Transfer the glass rod, or the tip of it to a tube full of ethanol and seal it well with Parafilm. If time permits, spool out some of the DNA and dry it down by blowing on it to remove most of the alcohol. Then gently stir it into a small amount of the NaCl solution in a clean test tube. It is also interesting to compare the viscosity of the DNA material to that of water and alcohol.

QUESTIONS

1. Name the two types of nucleic acids.
2. DNA is composed of only four different kinds of nucleotides. Name the three molecular parts of a nucleotide. Name the four different kinds of nitrogen-containing bases that occur in the nucleotides of DNA.
3. What is the purpose of homogenizing the thymus in the buffer?
4. Describe the appearance of the homogenate.
5. What color is the sediment (protein pellet)?
6. Where in the cell is the DNA located?
7. Explain the effect that detergent has on the cell membrane.
8. Why do you suppose you had to be careful in handling the preparation after lysing the nuclei? What lyses the cell and nucleus?
9. Were you surprised at the amount of DNA that you were able to isolate?
10. What protects the DNA? What precipitates the DNA?
11. Could you use the information from this activity to determine the amount of DNA per cell in calf thymus? Why or why not?

12. What did you like most about this activity? What did you like least?

SUGGESTED READING

Campbell, N.; Mitchell, L.; Reece, J. "Biology, Concepts and Connections"; 2nd ed. Addison Wesley Longman: Menlo Park, 1997, pp 171-181.

Chirikjian, J. "Biotechnology Theory and Techniques: Genetic Engineering Mutagenesis Separation Technology"; Vol. II. Jones and Bartlett: Sudbury, MA,1995, pp 3-13, 35-40.

Curtis, H. and Barnes. N.S. "Invitation to Biology"; 5th ed. Worth: New York, 1994.

DeSalle, Lindley 1997 pp 32-34

Edvotek, Inc. Resource Guide; "Spooling of Chromosomal DNA Experiment Module." EDVO-Kit # 107, West Bethesda, MD, 1997-98.

Johnson, G. "Biology, Visualizing Life"; Holt, Rinehart and Winston: Austin, pp 137-139, 1994.

Micklos, D.A.; Freyer, G.A. "DNA Science: A First Course in Recombinant DNA Technology";

Carolina Biological Supply Co., Burlington: NC. pp 48-50, 1990.

Moss, R.; Solomon, S. "American Biology Teacher". 1991. 53(7). 428-9.

Pennsylvania Biotechnology Association. Your World/Our World. 1996. 5(2). 6-7.

Pentz, L. "The Biolab Book"; 2nd Edition. Johns Hopkins University Press: Baltimore, 1989. pp. 83-87, 137-138.

Rasmussen, A.M.; Matheson, R.H. III. "A Sourcebook of Biotechnology Activities". National Association of Biology Teachers: North Carolina Biotechnology Center. 1990. pp 63-73.

Videodiscovery, Inc. "Anatomy and Physiology Laserdisc Teacher Directory", Bk 2, p 239. 1993.

Wegmann, L. "The Science Teacher". 1989. (9). 41-42.

Woodrow Wilson National Fellowship Foundation. "A Further Look at Biotechnology"; Princeton. 1993. pp 35-36, 47-52.

INSTRUCTOR NOTES

DNA Extraction from Calf Thymus

At the heart of modern biology lurks an extraordinary molecule called Deoxyribose Nucleic Acid (DNA). It is very long and thin and contains the genetic code needed to direct a cell's activities. One tends to take this miraculous substance for granted. It is such an unlikely molecule that it was almost ignored. DNA was isolated about a century ago by Friedrich Meischer (Pentz.1989). He extracted a new substance from the cells' nuclei that he called nucleic acid. Some of the content was protein, but part was a very peculiar acid, a polymer of four different subunits called nucleotides.

The discovery of DNA structure was a turning point in studies of inheritance. Meischer's discovery paved the way for many scientists whose work contributed to the understanding of DNA. Until then, no one had any idea of how the hereditary material is replicated prior to cell division. The Watson-Crick model suggested at once how this could be done (Starr, Taggart 1995).

DNA does not exist as a single strand but as two chains of nucleotides spiraling tightly around an imaginary axis to form a double helix. Deoxyribose sugar-phosphate backbones are on the outside of the helix, and the four different nitrogen bases are paired in the interior of the helix. Double strands are held together by hydrogen bonds between the complementary bases. The enzyme, DNA polymerase, ratchets along the parent strand to pick up the free-floating bases and attaches them to their complements (DeSalle, Lindley 1997). There is a crucial relationship between the two strands: where there is adenine (A) on one strand, it can only pair with thymine (T) on the other strand, and guanine (G) always pairs with cytosine (C). The complementary base pairs are like rungs on a spiral "rope ladder."

DNA is mainly found as tightly-coiled chromosomes in the eukaryotic cell nucleus. Human body cells each contain about two meters worth of DNA, and even though it is broken into 46 pieces, one for each chromosome, it is still a problem for the cell to get all this material neatly divided during mitosis. Generally the length of the DNA is related to the complexity of the organism. Chromosomes of many mammalian cells can contain DNA molecules that are over 50 million base pairs long. The complete DNA molecule of the human genome is much more complex. Within the haploid set of 23 chromosomes making up the human genome, there are approximately 3 billion nucleotide pairs. The diploid set found in each somatic cell has twice this number (Campbell, Mitchell, Reese 1997). The chromosomes in body cells are about 40 percent DNA and 60 percent proteins. Much of the DNA in human cells is noncoding. The protein serves as a scaffold around which the slender thread of DNA is tightly wound (Johnson, G.1994).

If the single DNA strand were stretched out in a straight line, it would be about 50 mm or 2 inches long. This is too long to fit inside a cell's nucleus. To keep it compact and more manageable, the DNA in eukaryotic cells is tightly wound around tiny particles made of very basic proteins called histones (Campbell, Mitchell, Reese 1997). The bends and twists in the "ladder" are all important to proper functioning of the DNA molecule. Other molecules involved in the transfer of genetic information migrate to the DNA (Sigma 1991).

Now that the structure of DNA has been studied for over 100 years and has basically been accepted, procedures have been devised to isolate almost pure DNA from its other components.

This project describes one method which helps students actually visualize the genetic material itself. After extracting the DNA, students can then subject the product to further testing in order to determine if DNA has been isolated, then run it on agarose gel to be analyzed by electrophoresis.

Many protocols for DNA extraction have been developed and tested. It is a fairly straightforward process. While there are easier and more economical methods, in my opinion this protocol produces the maximum yield of high quality DNA. A fresh commercial source of DNA must be inexpensive and relatively pure. Calf thymus cells contain a high concentration of DNA. This is one reason for using it as a source of chromosomal DNA. Also, it was easily available in my area. It is called "sweet bread" in our local meat markets. The thymus is a gland that is very large in immature animals. Since it functions as part of the immune system, it contains many white blood cells with large nuclei. Other sources include liver and salmon sperm.

Organic acids like DNA and bases like the histones bind together by their opposite charges, and a large enough quantity of any other charged substance (such as table salt) will interfere with this binding and allow the DNA to spring free. What happens when a long molecule like DNA is suddenly set free from its histones? Any solution of very long molecules has a number of striking changes in its physical properties. To begin with, the long molecules resist flow enough that the solution forms viscous, clotted masses which can be used as a rough indication of how long the molecules are. Alcohol, unlike water, is not an effective dissolving agent; therefore, charged molecules will tend to aggregate. One molecule of DNA is not visible by itself. By following the procedures in this investigation, DNA is visible because enough of the double helixes get entangled with each other to form a visible cable, like a strand of yarn spun from many wool fibers (Sigma 1991).

Even though DNA is strong, it is stiff and brittle and must be handled gently. Protocols for DNA isolation require measures to remove the RNA and positively charged, basic proteins. Degraded RNA usually contaminates the chromosomal DNA during extraction procedures. They have little tendency to spool onto the rod because they are too short and form finer, more uniform precipitates (Wegman, L.1989).

Most students visualize DNA as an impossibly small molecule. After all, it is inside a cell too small to be seen without a microscope and is further compressed into a nucleus inside that cell. It is not something that students normally expect to see and handle during a laboratory. Many thousands of thymus cells will be used for extraction; therefore, combinations of nuclei will allow the DNA to become visible.

Time Required

This lab generally requires more than one lab period. It is suggested that the instructor prepare the solutions and the homogenate in advance, store in refrigerator, and have students begin the procedure with the centrifuging process. If time is used efficiently, the remainder of the lab can be completed within a 50-minute period.

Group Size

Maximum efficiency and experience will be achieved with groups of three students.

Materials Needed (for group of 30 students)

(Recipes for all solutions follow in appendix.)

10 grams fresh or frozen calf thymus
kitchen blender
cheesecloth or centrifuge
6 50-mL graduated cylinders
ice cold ethanol
EDTA solution
sharp scalpel
6 solid glass rods
6 centrifuge tubes
timers
2 M NaCl solution
6 small test tubes
200 mL cold prep buffer
ice
glass pipettes
6 50-mL beakers
SDS solution
latex gloves

Safety, Handling, and Disposal

- Use caution when operating the centrifuge.
- Dispose of used reagents according to local ordinances.
- Wear latex gloves and goggles during the procedure.
- The laboratory surfaces should be very clean during all procedures used in this activity.
- Use thoroughly clean instruments and glassware. Rinse all equipment with isopropyl alcohol or acetone.
- Ethanol is highly flammable; use caution.

Points to Cover in the Pre-Lab Discussion

Stress safety and the basic results which can be expected when performing DNA extraction. (See Procedural Tips and Suggestions.)

Procedural Tips and Suggestions

This laboratory may appear to be too difficult for the average high school Biology I student. However, once the instructor becomes comfortable with the techniques, one should be able to effectively guide students through the process.

When DNA extractions are performed, three basic results can be expected:

1. No DNA,
2. DNA appears fluffy, which means it has sheared in the extraction process, or
3. DNA appears as thin threads.

It is critical to follow all directions very carefully and be sure all surfaces and equipment being used are very clean.

Sample Results

When unwound, DNA is a long, thin, sticky fiber, similar in texture to mucous. If the DNA is of very high purity, it will be pure white or, to be more accurate, somewhat iridescent, like an opal. Take time to examine the product closely in different lighting conditions.

Plausible Answers to Student Questions

How do you know if your product is DNA? You could run it on a gel and compare it to standard markers. This process affirms the physical intactness of the extracted DNA in the resulting product.

Extensions and Variations

a. Design your own experiment to test the effects of different experimental conditions on the DNA; such as heat, different concentrations of the salt solution, and testing the purity using spectrophotometric tests. (See Solomon and Moss.)

b. Re-dissolve spooled DNA, then analyze it by gel electrophoresis. Having physically intact DNA can be assessed by analysis using 0.8% agarose gel electrophoresis. The spooled DNA must first be resuspended.

Directions for preparing the agarose and for setting up the equipment can be obtained from most lab manuals and other specialty publications (Chirikjian 1995, Edvotek 1991).

1. Basically all that needs to be done is to let the glass rod sit inside a test tube and add 2 mL of the resuspension solution to the tube. Allow DNA to resuspend for 30 minutes.
2. Add 40 microliters of the DNA solution to a 1.5 mL Eppendorf test tube. Mix gently with 5 microliters of gel loading solution.
3. Run on agarose gel and stain with methylene blue to visualize bands.
4. Destain in water until DNA bands can be visualized.
5. Samples can be run against standard markers to determine the length of sample in base pairs.

c. Try to denature the spooled DNA in chemicals such as methanol, hydrochloric acid, or sodium hydroxide.

APPENDIX

Sources of Materials

1. Calf thymus may be known to your butcher as "sweetbreads." Order approximately one week before needed. Insist on fresh thymus or make sure it has been frozen in the packing house immediately after slaughtering the animal. Do not allow to thaw, keep frozen; could keep as long as 4 years if properly handled.

2. Most of the required chemicals can be obtained from:

Sigma Chemical Co. {As well as other suppliers}

P.O. Box 14508

St. Louis, MO 63178

(800) 325-3010

3. Solvents can be obtained from:

Fisher Scientific {As well as other suppliers}

2761 Walnut Ave

Tustin, CA 92680

(714) 832-9800

For those whose budgets are extremely tight or who have difficulty obtaining chemicals, the activity works with the following SUBSTITUTIONS. **In each case, substitute the same weighed or measured amounts as called for in the directions.**

Prep Buffer: table sugar instead of sucrose
Epsom salts instead of Magnesium chloride
buffered aspirin instead of Tris-HCl
EDTA may be omitted
Liquid Joy or Dawn dish washing detergent instead of SDS
2 M NaCl: kosher salt (not iodized table salt)
alcohol: rubbing alcohol

Preparation of Solutions

Prep Buffer:
57 g sucrose
3.1 g $MgCl_2 \cdot 6H_2O$
0.6 g Tris-HCl
Make up to 500 mL with distilled water. Adjust the pH to 7.5 with 0.1 N HCl. (The solution will normally start out with a high pH.) Store refrigerated. May be frozen for long-term storage. Keeps for at least a week refrigerated; discard if cloudy or moldy.

EDTA:
0.72 g disodium EDTA • $2H_2O$
Make up to 250 mL with distilled water. Adjust pH to 7.5 with 0.1 N NaOH; this will help dissolve the EDTA. Check pH when completely dissolved. Store at room temperature. Keeps indefinitely.

SDS:
25 g sodium dodecyl sulfate (= sodium lauryl sulfate, SLS)
Make up to 250 mL with distilled water, dissolve gently, avoiding excessive foaming. Store refrigerated. Keeps indefinitely. (Will freeze solid in the refrigerator; simply thaw before use.)

2 M NaCl:
29.2 g sodium chloride
Make up 250 mL with distilled water. Store at room temperature. Keeps indefinitely.

Alcohol:
Normally, pure ethyl alcohol (100%) is used in this procedure. However, perfectly good results have been obtained with isopropyl alcohol. Even the 70% isopropanol sold as "rubbing alcohol" seems satisfactory. These alcohols can also be used to clean the glassware before the lab, although acetone will evaporate faster. Wait to use the glassware until it no longer smells of the alcohol or acetone.

References

Bear, Jenifer. Biotechnology Workshop sponsored by NABT, EDVOTEK, Inc. and Georgetown University. NSTA Convention, Kansas City, MO. Oct. 1990.

Campbell, N. "Biology". Fourth Edition. The Benjamin/Cummings Publishing Company, Inc. Menlo Park. 1996, pp 85, 377.

Campbell, N.; Mitchell, L.; Reece, J. "Biology, Concepts and Connections"; 2nd ed. Addison Wesley Longman: Menlo Park, 1997, pp 171-181.

Chirikjian, J. "Biotechnology Theory and Techniques: Genetic Engineering Mutagenesis Separation Technology"; Vol. II. Jones and Bartlett: Sudbury, MA,1995, pp 3-13, 35-40.

Curtis, H. and Barnes. N.S. "Invitation to Biology"; 5th ed. Worth: New York, 1994.

DeSalle, R.; Lindley, D. "The Science of Jurassic Park and The Lost World." Basic Books, New York. 1997. pp 32-34.

Edvotek, Inc. Resource Guide; "Spooling of Chromosomal DNA Experiment Module". EDVO-Kit # 107, West Bethesda, MD, 1997-98.

Johnson, G. “Biology, Visualizing Life”; Holt, Rinehart and Winston: Austin, pp 137-139, 1994.

Micklos, D.A.; Freyer, G.A. “DNA Science: A First Course in Recombinant DNA Technology”; Carolina Biological Supply Co., Burlington: NC. pp 48-50, 1990.

Moss, R.; Solomon, S. “American Biology Teacher”. 1991. 53(7). 428-9.

Pennsylvania Biotechnology Association. Your World/Our World. 1996. 5(2). 6-7.

Pentz, L. “The Biolab Book”; 2nd Edition. Johns Hopkins University Press: Baltimore, 1989. pp. 83-87, 137-138.

Rasmussen, A.M.; Matheson, R.H. III. “A Sourcebook of Biotechnology Activities”. National Association of Biology Teachers: North Carolina Biotechnology Center. 1990. pp 63-73.

Schmeck, H. “Blazing a Genetic Trail”. Howard Hughes Medical Institute. Bethesda. 1991. pp. 50-51.

Sigma Molecular Biology. “DNA Spooling Educational Kit”. Technical Bulletin No. ED100. St. Louis, Nov. 1991.

Starr, C.; Taggart, R. “Biology. The Unity and Diversity of Life”. Seventh Edition. Wadsworth: Belmont, CA. 1995. pp 212-217.

Videodiscovery, Inc. “Anatomy and Physiology Laserdisc Teacher Directory”, Bk 2, Seattle, 1993. p 239.

Videodiscovery, Inc. “Genetics Laserdisc Teacher Directory”, Bk 2, Seattle, 1995.

Wegmann, L. “The Science Teacher”. 1989. (9). 41-42.

Woodrow Wilson National Fellowship Foundation. “A Further Look at Biotechnology”; Princeton. 1993. pp 35-36, 47-52.