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SIGMA®

ProductInformation

DEOXYRIBONUCLEIC ACID (DNA) SPOOLING EDUCATIONAL KIT

Product Number **D 8666**
Technical Bulletin ED-100

TECHNICAL BULLETIN

Product Description
Deoxyribonucleic acid, usually abbreviated as DNA, is a long double-helical molecule containing genetic information. DNA is mainly found as tightly-coiled chromosomes in the nucleus of the cell, or as more loosely coiled strands in a bacterial cell which has no nucleus. If you uncoiled all the DNA in one human cell, the filament which is invisible to the human eye would measure about 1.5 meters. If it were multiplied a thousand times, it would be 1500 meters long (almost a mile), but still thinner than a fine hair.

A DNA molecule does not exist in humans as a long loose Arope@, but is coiled and twisted much like an extra-long telephone cord that has not been untangled for years. The thick twisted bundles of DNA are the chromosomes that can be seen with a powerful microscope. DNA is a double-stranded molecule. Each strand is composed of an ordered combination of four nucleotides, each nucleotide consisting of a purine or pyrimidine base (adenine, guanine, thymine or cytosine) associated with a deoxyribose sugar molecule and a phosphate group. A base in one strand can form weak, but significant, hydrogen bonds to a complementary base in the other strand (or even to a complementary base somewhere in the same strand). These base-pairs are like rungs in a spiral Arope ladder@. The bends and twists in this ladder all are important to the functioning of the DNA molecule. The folded shape is so complex and sensitive that other molecules involved in the transfer of genetic information migrate to the DNA, rather than the other way around. If the structure of DNA is altered, so is its function. If it were to completely uncoiled, it would no longer function properly in human cells.

The sequence of bases in a DNA molecule from any organism is a code for the synthesis of each and every molecule of that organism. In general, the length of the DNA is related to the complexity of the creature with which it is associated. A virus DNA may have only 3000 base pairs, a bacterium has about 3 million base pairs. Mammals, including humans, have 3-5 billion base pairs.

Extracting DNA from cells is not an easy task. The cell walls and nuclear membranes must by lysed (broken) so that the DNA molecules can be dissolved into a solvent. The DNA must then be separated from cell debris, substructures, and other molecules. Cells also contain a variety of enzymes, some of which attack and destroy nucleic acids. The DNA must be protected from these nucleases as it is being isolated.

From a practical viewpoint, a commercial source of DNA must be inexpensive and rich in DNA. Although sirloin steak contains DNA, it is extremely expensive and contains lipids, saccharides, and protein material which must be discarded. Not practical! Two excellent sources of DNA are sperm cells and thymus glands of very young animals (their function is the development of immune systems; they become dysfunctional in older animals). Since these cells contain almost nothing but DNA, this makes the isolation procedure much simpler. Calf thymus DNA is available from Sigma, but thymus glands are very small; often called Asweetbreads@, they are a restaurant delicacy priced like steak. By comparison, salmon sperm is more readily available.

The isolation process for DNA is straightforward, but too long for most high school class periods, and highly dependent on good laboratory skills. Most college biology laboratories have procedures for isolation of

DNA from yeast or bacterial strains. These procedures involve lysing cell walls, centrifuging fragments, and carefully separating the DNA from the proteins, carbohydrates, lipids, and other molecules present. The DNA solution must be buffered (that is, protected from extreme changes in acidity). The long chain must be untangled using substances which disrupt the cross-links between coils, so the twisted rope can relax. DNA is soluble in water, but not in alcohol. Alcohol can denature macromolecules, i.e., push them out of solution and disrupt the cross-linking that maintains the coiled structure.

This kit allows you to take the final step in the isolation of DNA and to have the success of seeing a macromolecule without the often frustrating problems associated with cell lysis, etc. The addition of alcohol to a solution of DNA will precipitate it, allowing you to retrieve strands of DNA with a glass rod. One molecule of DNA is not visible by itself, but if enough molecules are entangled with each other, you will be able to see the strands of DNA.

Components

Kit is sufficient for 25 student demonstrations

- DNA Solution, Product No. D 1666, 25 ml
  DNA (salmon testes), 1 mg/ml, in 0.01 M Tris-HCl, pH 8.0 at 25 °C, and 0.001 M EDTA

- Sodium Acetate 3 M Solution, 25 ml
  Product No. S 7899 pH 5.2 at 25 °C

Materials Required but Not Provided

Isopropanol, 100 ml or ethanol (95-100% w/v),
200 ml Test tubes, glass or plastic, 10 ml (or other suitable clear containers) (25 total)
Pipetting devices for the accurate delivery of volumes required for the assay
Glass stirring rods or glass pipets (25 total)
Petri dishes, plastic or glass (25 total)

Precautions and Disclaimer

Sigma DNA reagents are for laboratory use only. Not for drug, household or other uses. Normal precautions exercised in handling laboratory reagents should be followed. Do NOT pipet by mouth. Refer to Material Safety Data Sheets for any updated risk, hazard, or safety information.

Storage/Stability

Reagents are stable indefinitely when stored in the freezer at –20 °C.

Procedure

1. Bring the DNA solution, Product No. D 1666 and sodium acetate 3 M solution, Product No. S 7899, to room temperature.
2. Pipette 1 ml of DNA solution into each test tube.
3. Slowly add 1 ml of sodium acetate 3 M solution, to the DNA solution while tilting tube and letting the sodium acetate run down side of tube. Mix by gently swirling.
4. Slowly add 2 ml of isopropanol and observe the layering. NOTE: If isopropanol is not available, use 4 ml of ethanol. Results will be the same.
5. With a glass stirring rod or glass pipet, gently mix the aqueous and organic phases. DNA will start to precipitate. Gently spool the DNA around the glass rod.
6. Carefully remove the DNA and place on a petri dish or other suitable surface and let dry overnight. The DNA you have isolated is ready for storage or examination under a microscope. It may also be used for other laboratory projects.

References

You may enjoy reading more about what DNA does and how genetic codes can help anticipate development of disease, answer legal questions of identity, solve crimes and more. Most current biology and biochemistry textbooks contain basic background information about DNA technology. Some of the information above was derived from the following books. Their excellent illustrations would add a great deal to your understanding of DNA. These are available from Sigma Chemical Company; see our catalog for more information and additional book listings.

BIOKIT: A Journey to Life, J. de Rosnay, Adama Books, New York, NY, 1984.
A very entertaining introduction to the mechanism of DNA/RNA and protein synthesis. Even has a Biodisc which allows you to assemble your own proteins! Appropriate for high school or college; clever cartoon illustrations add considerable appeal. (Product No. BIO-1)

DNA SCIENCE: A First Course in Recombinant DNA Technology, D.A. Micklos and G.A. Freyer, Carolina Biological Supply Co., Burlington, NC, 1990.
Intended for a college biology class: a special unit about DNA technology. Good background information with lab experiments included in the book. Could be used in advanced high school special topics science course. (Product No. D4667)

BIOCHEMISTRY, 4th ed., L. Stryer, W.H. Freeman & Co., New York, NY, 1995.
The fourth edition is a challenging reappraisal of the discipline by an active research scientist and teacher. Readers will see how dramatically the field has been transformed by recombinant DNA technology.

The next page of the bulletin has a student report form which may be photocopied for classroom usage.

MAM 02/02

STUDENT REPORT FORM: SPOOLING DNA

NAME: ___________________________________

CLASS HOURS: ___________________________

INSTRUCTOR: ____________________________

The nucleic acid you will see today is deoxyribonucleic acid (DNA). As you perform the investigation note your findings.

1. When alcohol is added to the DNA/Sodium Acetate Solution which liquid formed the top layer?

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2. What was the purpose of the alcohol?

____

3. What did the DNA look like?

   a. Describe the solution of DNA (color/viscosity)

   ____

   b. Describe it as it came out of the solution (color, etc.)

   ____

   c. Describe the DNA after it has been exposed to air to dry.

   ____

4. What did you think DNA would look like before you did this experiment? Were you surprised?

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